

68 REQUEST FORM FOR DIVISIONAL APPLICATION UNDER 37 C.F.R. \$1.60

08/819669

72192 U.S. PTO



03/17/97

Document Number: LUD-5253.5 DIV

03/17/97
Anticipated Classification of this Application

Class: Subclass:
Prior Application: Serial No.:
Examiner: Art Unit:

"Express Mail" mailing label No. EH473512443US

Date of Deposit March 17, 1997
I hereby certify that this paper and/or fee is being deposited with the United States Postal Service as first class mail service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Box Patent Application, Washington, D.C. 20231

By Annette Abdelleh
(print name)

Annette Abdelleh
(signature)

Commissioner of Patents and Trademarks
Box **PATENT APPLICATION**
Washington, D.C. 20231

This is a Request for filing a
() Continuation-in-Part () Continuation (X) Divisional Application
under 37 CFR 1.60 of prior application Serial No. 08/142,368, filed on May 2,
1994 entitled TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR REJECTION ANTIGENS
AND USES THEREOF by inventors:

Thierry BOON Belgium
(Full Name of Inventor) (Citizenship)
Residence: Brussels, Belgium
(City and State)
Post Office Address: Ave. Hippocrate, 74, UCL 7459
Brussels B-1200, Belgium
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08/819669-1034780

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Patrick CHOMEZ Belgium
 (Full Name of Inventor) (Citizenship)
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 (City, State and Zip Code)

Catia TRAVERSARI Italy
 (Full Name of Inventor) (Citizenship)
 Residence: Milan, Italy
 (City and State)
 Post Office Address: Sesto S. Giovanni, I-20099
Milan, Italy
 (City, State and Zip Code)

The above identified prior application in which no payment of the issue fee, abandonment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application. (Note: 37 CFR 1.60 may be used for applications where the prior application is not to be abandoned).

1. ☐ Enter all amendments in the prior application.
2. ☒ Preliminary Amendment

The filing fee is calculated on the basis of the claims existing in the prior application as amended at 1 and 2 above.

For	Number <u>Filed</u>	Number <u>Extra</u>	Rate	Basic fee (\$770.00)
Total Claims.....	6 -20		x \$22.00	= \$-0-
Independent Claims.	1 -3		x \$80.00	= \$-0-

☐ Multiple Dependent Claims - where applicable (\$260.00).

(Reduction by 1/2 for filing by small entity. If applicable, verified statement must be attached).

TOTAL FILING FEE \$ 770.00

3. ☒ A check in the amount of \$770.00 is enclosed to cover the filing fee. In the event the enclosed check is unacceptable and/or insufficient to cover the required fee, or omitted, please charge to Account No. 06-0530.

4. () A new oath or declaration is included since this application is a continuation-in-part which discloses and claims additional matter.

5. () Amend the specification by inserting before the first line the sentence: This application is a () continuation-in-part, () continuation, (X) divisional, of application Serial No. 08/142,368, filed May 2, 1994, now U. S. Patent Application No. _____.

6. () A verified statement claiming small entity status is enclosed. (Necessary even if a statement was filed in the prior application).

7. () Priority of application Serial No. _____ filed
, 19 ____ is claimed under 35 U.S.C. §119.

8. (X) The prior application is assigned of record to LUDWIG INSTITUTE FOR CANCER RESEARCH, 1345 AVENUE OF THE AMERICAS, NEW YORK, NY 10105

9. () A petition and extension fee has been filed to extend the term in the pending prior Application until _____. A copy of the petition for extension of time in the prior Application is attached.

10. (X) The power of attorney in the prior application is to:
Peter F. Felfe, Reg. No. 20,297; John E. Lynch, Reg. No. 20,940; Alfred H. Hemingway, Jr., Reg. No. 26,736; Vincent M. Fazzari, Reg. No. 26,879; Norman D. Hanson, Reg. No. 30,946; F. Brice Faller, Reg. No. 29,532; Andrew L. Tiajolloff, Reg. No. 31,575; John P. Luther, Reg. No. 32,261; John A. Bauer, Reg. No. 32,554; Edward P. Kelly; and Patricia A. Pasqualini, Reg. No. 34,894 my attorneys with full power of substitution and revocation; and Walter G. Weissenberger, Reg. No. 17,344.


11. () Also enclosed: Request for _____-Month Extension of Time

Address all future communications to:

Norman D. Hanson, Esq.
FELFE & LYNCH
805 Third Avenue
New York, New York 10022

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application, be it either this application or a prior application in the same file wrapper, the Patent and Trademark Office may provide similar information or access to all the other applications in the same file wrapper.

Dated: March 17, 1997



Norman D. Hanson
Reg. No. 30,946

08/819669



03/17/97

Attorney Docket: LUD-5253.5 DIV-JEL/NDH

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Thierry Boon-Falleur et al. Date: March 17, 1997
 Serial No.: To Be Assigned Filing Date: Herewith
 Group Art Unit: Unknown Examiner: Unknown

Honorable Commissioner of Patents
 and Trademarks
 Washington, D.C. 20231

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By Annette Abdelleh
 (print name)

Annette Abdelleh
 (signature)

PRELIMINARY AMENDMENT

Prior to examination, please amend this divisional application as follows:

IN THE SPECIFICATION:

Page 1, line 1, after "1991" add --now U.S. Patent No. 5,342,774--;
 line 5, change "764,364" to --764,365--;
 line 6, after "1991" add --now abandoned--.

Page 4, line 16, change "Fearson" to --Fearon--.

IN THE CLAIMS:

Cancel claims 1-172 without prejudice.

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Please add new claims 173-178 which follow:

173. An isolated tumor rejection antigen precursor protein, said protein being a non tum-antigen.

174. The isolated tumor rejection antigen precursor of claim 173, wherein said tumor rejection antigen precursor is a human protein.

175. The isolated tumor rejection antigen precursor of claim 174, which has an amino acid sequence encoded by SEQ ID NO: 8.

176. The isolated tumor rejection antigen precursor of claim 174, wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary nucleotide sequence of which hybridizes to SEQ ID NO: 8, under stringent conditions.

177. The isolated tumor rejection antigen precursor of claim 176, encoded by a nucleic acid molecule which has a nucleotide sequence selected from the group consisting of SEQ ID NO: 9 through SEQ ID NO: 25.

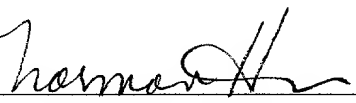
178. The isolated tumor rejection antigen precursor of claim 177, encoded by the nucleic acid molecule having the nucleotide sequence set forth in SEQ ID NO: 11 or SEQ ID NO: 12.

REMARKS

Entry of the foregoing preliminary amendment is requested. The claims correspond to those claims included in Group V of the restriction requirement of September 27, 1995, in the parent case, which has now been allowed.

Respectfully submitted,

FELFE & LYNCH

By 
Norman D. Hanson
Reg. No. 30,946

NDH:aa

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25/10/95 09:06:50



Attorney Docket: LUD-5253.5 DIV-JEL/NDH

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Thierry Boon-Falleur et al. Date: March 17, 1997
Serial No.: To Be Assigned Filing Date: Herewith
Group Art Unit: Unknown Examiner: Unknown

Honorable Commissioner of Patents
and Trademarks
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By Annette Abdelleh
(print name)

Annette Abdelleh
(signature)

LETTER RE:
TRANSFER OF SEQUENCE INFORMATION

The above-referenced application contains sequence information. The sequences are identical to those filed in Serial No. 08/142,368. The CRF was mailed on January 26, 1995. It is requested that the CRF submitted on January 26, 1995 be transferred to this case. A paper copy of the sequence information is attached hereto.

The undersigned hereby attests to the absence of new matter in these submissions.

Respectfully submitted,

FELFE & LYNCH

By Norman D. Hanson
Norman D. Hanson
Reg. No. 30,946

NDH:aa
Attachment

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(1) GENERAL INFORMATION:

- (i) APPLICANTS: Boon-Falleur, Thierry; Van der Bruggen, Thierry; Van den Eynde, Benoit; Van Pel, Aline; De Plaen, Etienne; Lurquin, Christophe; Chomez, Patrick; Traversari, Catia
- (ii) TITLE OF INVENTION: Tumor Rejection Antigen Precursors, Tumor Rejection Antigens and Uses Thereof
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Felfe & Lynch
(B) STREET: 805 Third Avenue
(C) CITY: New York City
(D) STATE: New York
(F) ZIP: 10022
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/142,368
(B) FILING DATE: 02-MAY-1994
(C) CLASSIFICATION: 435
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US92/04354
(B) FILING DATE: 22-MAY-1992
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/807,043
(B) FILING DATE: 12-DECEMBER-1991
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/764,364
(B) FILING DATE: 23-SEPTEMBER-1991
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/728,838
(b) FILING DATE: 9-JULY-1991
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/705,702
(B) FILING DATE: 23-May-1991
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hanson, Norman D.
(B) REGISTRATION NUMBER: 30,946
(C) REFERENCE/DOCKET NUMBER: LUD 5253.4-US

RECEIVED 034797

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 688-9200
(B) TELEFAX: (212) 838-3884

- (2) INFORMATION FOR SEQUENCE ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT GAAGATCCTG 60
ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT CAGCCAATGA GCTTACTGTT 120
CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG AAGTTTGTGCA AGTTCCGCCT ACAGCTCTAG 180
CTTGTGAATT TGTACCCTTT CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC 240
CCCCCTCCCA CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT 300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG CATGCATTGT 360
GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG CTAGCTTGCG ACTCTACTCT 420
TATCTTAACT TAGCTCGGCT TCCTGCTGGT ACCCTTTGTG CC 462

- (2) INFORMATION FOR SEQUENCE ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT 48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly
5 10 15
GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA 96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu
20 25 30
GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA 144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr
35 40 45
AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG 192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln
50 55 60
TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC 240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser

[illegible]

(2) INFORMATION FOR SEQUENCE ID NO: 3:

GCATGCAGTT	GCAAAGCCCA	GAAGAAAGAA	ATGGACAGCG	GAAGAAGTGG	TTGTTTTTTT	60
TTCCCCCTTCA	TTAATTTTCT	AGTTTTTtagt	AATCCAGAAA	ATTTGATTTT	GTTCTAAAGT	120
TCATTATGCA	AAGATGTCAC	CAACAGACTT	CTGACTGCAT	GGTGAAC TTT	CATATGATAC	180
ATAGGATTAC	ACTTG TACCT	GTTAAAAATA	AAAGTTTGAC	TTGCATAC		228

(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1365 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT 50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT 100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG 150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT 200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA 250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT 300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCTTT GCTCTCCCAG 350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG 400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT 450
ACCCTTTGTG CC 462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA 504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG 546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC 588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC 630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC 672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG 714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC 756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT 798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA 840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA 882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT 924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT 966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG 1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT 1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAG GAG GAG 1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT 1134
TAG 1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG 1187
TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA 1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT 1287
CTGACTGCAT GGTGAACCTT CATATGATAC ATAGGATTAC ACTTGACCT 1337
GTTAAAAATA AAAGTTTGAC TTGCATAC 1365

462TCTGCTGGT

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACCACAGGAG	AATGAAAAGA	ACCCGGGACT	CCCAAAGACG	CTAGATGTGT	50
GAAGATCCTG	ATCACTCATT	GGGTGTCTGA	GTTCTGCGAT	ATTCATCCCT	100
CAGCCAATGA	GCTTACTGTT	CTCGTGGGGG	GTTTGTGAGC	CTTGGGTAGG	150
AAGTTTTGCA	AGTTCCGCCT	ACAGCTCTAG	CTTGTGAATT	TGTACCCTTT	200
CACGTAAAAA	AGTAGTCCAG	AGTTTACTAC	ACCCTCCCTC	CCCCCTCCCA	250
CCTCGTGCTG	TGCTGAGTTT	AGAAGTCTTC	CTTATAGAAG	TCTTCCGTAT	300
AGAACTCTTC	CGGAGGAAGG	AGGGAGGACC	CCCCCCTTTT	GCTCTCCCG	350
CATGCATTGT	GTCAACGCCA	TTGCACTGAG	CTGGTCGAAG	AAGTAAGCCG	400
CTAGCTTGCG	ACTCTACTCT	TATCTTAACT	TAGCTCGGCT	TCCTGCTGGT	450
ACCCTTTGTG	CC				462
ATG TCT GAT	AAC AAG AAA	CCA GAC AAA	GCC CAC AGT	GGC TCA	504
GGT GGT GAC	GGT GAT GGG	AAT AGG TGC	AAT TTA TTG	CAC CGG	546
TAC TCC CTG	GAA GAA ATT	CTG CCT TAT	CTA GGG TGG	CTG GTC	588
TTC GCT GTT	GTC ACA ACA	AGT TTT CTG	GCG CTC CAG	ATG TTC	630
ATA GAC GCC	CTT TAT GAG	GAG CAG TAT	GAA AGG GAT	GTG GCC	672
TGG ATA GCC	AGG CAA AGC	AAG CGC ATG	TCC TCT GTC	GAT GAG	714
GAT GAA GAC	GAT GAG GAT	GAT GAG GAT	GAC TAC TAC	GAC GAC	756
GAG GAC GAC	GAC GAC GAT	GCC TTC TAT	GAT GAT GAG	GAT GAT	798
GAG GAA GAA	GAA TTG GAG	AAC CTG ATG	GAT GAT GAA	TCA GAA	840
GAT GAG GCC	GAA GAA GAG	ATG AGC GTG	GAA ATG GGT	GCC GGA	882
GCT GAG GAA	ATG GGT GCT	GGC GCT AAC	TGT GCC T		916
GTGAGTAACC	CGTGGTCTTT	ACTCTAGATT	CAGGTGGGGT	GCATTCTTTA	966
CTCTTGCCCA	CATCTGTAGT	AAAGACCACA	TTTTGGTTGG	GGGTCATTGC	1016
TGGAGCCATT	CCTGGCTCTC	CTGTCCACGC	CTATCCCCGC	TCCTCCCATC	1066
CCCCACTCCT	TGCTCCGCTC	TCTTTCCCTT	TCCCACCTTG	CCTCTGGAGC	1116
TTCACTCCAT	CCTGCTCTGC	TCCCTTTCCC	CTTTGCTCTC	CTTGCTCCCC	1166
TCCCCCTCGG	CTCAACTTTT	CGTGCCTTCT	GCTCTCTGAT	CCCCACCCTC	1216
TTCAAGCTTC	CCCATTGCT	CCTCTCCCGA	AACCCTCCCC	TTCTGTTC	1266
CCTTTTCGCG	CCTTTTCTTT	CCTGCTCCCC	TCCCCCTCCC	TATTTACCTT	1316
TCACCAGCTT	TGCTCTCCCT	GCTCCCCCTC	CCCTTTTGCA	CCTTTTCTTT	1366
TCCTGCTCCC	CTCCCCCTCC	CCTCCCTGTT	TACCCTTCAC	CGCTTTTCCT	1416
CTACCTGCTT	CCCTCCCCCT	TGCTGCTCCC	TCCCTATTG	CATTTTCGGG	1466
TGCTCCTCCC	TCCCCCTCCC	CCTCCCTCCC	TATTTGCATT	TTGCGGTGCT	1516
CCTCCCTCCC	CCTCCCCAGG	CCTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	1566
TTGGTTTTTC	GAGACAGGGT	TTCTCTTTGT	ATCCCTGGCT	GTCCTGGCAC	1616
TCACCTCTGA	GACCAGGCTG	GCCTCAAAC	CAGAAATCTG	CCTGCCTCTG	1666
CCTCCCAAAT	GCTGGGATTA	AAGGCTTGCA	CCAGGACTGC	CCCAGTGCAG	1716
GCCTTTCTTT	TTTCTCCTCT	CTGGTCTCCC	TAATCCCTTT	TCTGCATGTT	1766
AACTCCCCTT	TTGGCACCTT	TCCTTTACAG	GACCCCTCC	CCCTCCCTGT	1816
TTCCCTTCCG	GCACCCTTCC	TAGCCCTGCT	CTGTTCCCTC	TCCCTGCTCC	1866
CCTCCCCCTC	TTTGCTCGAC	TTTTAGCAGC	CTTACCTCTC	CCTGCTTTCT	1916
GCCCCGTTCC	CCTTTTTTGT	GCCTTTCCCTC	CTGGCTCCCC	TCCACCTTCC	1966
AGCTCACCTT	TTTGTGTTGT	TGGTTGTTTG	GTTGTTTGGT	TTGCTTTTTT	2016
TTTTTTTTTT	GCACCTTGTT	TTCCAAGATC	CCCCTCCCCC	TCCGGCTTCC	2066
CCTCTGTGTG	CCTTTCCTGT	TCCCTCCCCC	TCGCTGGCTC	CCCCTCCCTT	2116

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TCTGCCTTTC	CTGTCCCTGC	TCCCTTCTCT	GCTAACCTTT	TAATGCCTTT	2166
CTTTTCTAGA	CTCCCCCTC	CAGGCTTGCT	GTTTGCTTCT	GTGCACTTTT	2216
CCTGACCCTG	CTCCCCCTCC	CCTCCCAGCT	CCCCCTCTT	TTCCCACCTC	2266
CCTTTCTCCA	GCCTGTCACC	CCTCCTTCTC	TCCTCTCTGT	TTCTCCCCT	2316
TCCTGCTTCC	TTTACCCCTT	CCCTCTCCCT	ACTCTCCTCC	CTGCCTGCTG	2366
GACTTCCTCT	CCAGCCGCCC	AGTTCCCTGC	AGTCCTGGAG	TCTTTCCTGC	2416
CTCTCTGTCC	ATCACTTCCC	CCTAGTTTCA	CTTCCCTTTC	ACTCTCCCCT	2466
ATGTGTCTCT	CTTCCCTATCT	ATCCCTTCCT	TTCTGTCCCC	TCTCCTCTGT	2516
CCATCACCTC	TCTCCTCCCT	TCCCTTTCCT	CTCTCTTCCA	TTTTCTTCCA	2566
CCTGCTTCTT	TACCCTGCCT	CTCCCATTGC	CCTCTTACCT	TTATGCCCCAT	2616
TCCATGTCCC	CTCTCAATTC	CCTGTCCCAT	TGTGCTCCCT	CACATCTTCC	2666
ATTTCCCTCT	TTCTCCCTTA	GCCTCTTCTT	CCTCTTCTCT	TGTATCTCCC	2716
TTCCCTTTGC	TTCTCCCTCC	TCCTTTCCCC	TTCCCTATG	CCCTCTACTC	2766
TACTTGATCT	TCTCTCCTCT	CCACATAACC	TTTTTCCTTT	CCACCCTGCC	2816
CTTTGTCCCC	AGACCCTACA	GTATCCTGTG	CACAGGAAGT	GGGAGGTGCC	2866
ATCAACAACA	AGGAGGCAAG	AAACAGAGCA	AAATCCCAAA	ATCAGCAGGA	2916
AAGGCTGGAT	GAAAATAAGG	CCAGGTTCTG	AGGACAGCTG	GAATCTAGCC	2966
AAGTGGCTCC	TATAACCCTA	AGTACCAAGG	GAGAAAGTGA	TGGTGAAGTT	3016
CTTGATCCTT	GCTGCTTCTT	TTACATATGT	TGGCACATCT	TTCTCAAATG	3066
CAGGCCATGC	TCCATGCTTG	GCGCTTGCTC	AGCGTGGTTA	AGTAATGGGA	3116
GAATCTGAAA	ACTAGGGGCC	AGTGGTTTGT	TTGGGGGACA	AATTAGCACG	3166
TAGTGATATT	TCCCCCTAAA	AATTATAACA	AACAGATTCA	TGATTTGAGA	3216
TCCTTCTACA	GGTGAGAAGT	GGAAAAATTG	TCACTATGAA	GTTCTTTTTA	3266
GGCTAAAGAT	ACTTGGAACC	ATAGAAGCGT	TGTTAAAATA	CTGCTTTCTT	3316
TTGCTAAAAT	ATTCTTTCTC	ACATATTTCAT	ATTCTCCAG		3355
GT GTT CCT	GGC CAT CAT	TTA AGG	AAG AAT GAA	GTG AAG TGT	3396
AGG ATG ATT	TAT TTC TTC	CAC GAC	CCT AAT TTC	CTG GTG TCT	3438
ATA CCA GTG	AAC CCT AAG	GAA CAA	ATG GAG TGT	AGG TGT GAA	3480
AAT GCT GAT	GAA GAG GTT	GCA ATG	GAA GAG GAA	GAA GAA GAA	3522
GAG GAG GAG	GAG GAG GAA	GAG GAA	ATG GGA AAC	CCG GAT GGC	3564
TTC TCA CCT	TAG				3576
GCATGCAGGT	ACTGGCTTCA	CTAACCAACC	ATTCCTAACA	TATGCCTGTA	3626
GCTAAGAGCA	TCTTTTAAAA	AAATATTATT	GGTAAACTAA	ACAATTGTGA	3676
TCTTTTAAAA	TTAATAAGTA	TTAAATTAAT	CCAGTATACA	GTTTAAAGAA	3726
CCCTAAGTTA	AACAGAAGTC	AATGATGTCT	AGATGCCTGT	TCTTTAGATT	3776
GTAGTGAGAC	TACTTACTAC	AGATGAGAAG	TTGTTAGACT	CGGGAGTAGA	3826
GACCAGTAAA	AGATCATGCA	GTGAAATGTG	GCCATGGAAA	TCGCATATTG	3876
TTCTTATAGT	ACCTTTGAGA	CAGCTGATAA	CAGCTGACAA	AAATAAGTGT	3926
TTCAAGAAAG	ATCACACGCC	ATGGTTCACA	TGCAAATTAT	TATTTTGTCTG	3976
TTCTGATTTT	TTTCATTTCT	AGACCTGTGG	TTTTAAAGAG	ATGAAAATCT	4026
CTTAAAAATTT	CCTTCATCTT	TAATTTTCCT	TAACCTTAGT	TTTTTTCACT	4076
TAGAATTCAA	TTCAAATTCT	TAATTCAATC	TTAATTTTAA	GATTTCTTAA	4126
AATGTTTTTT	AAAAAAAATG	CAAATCTCAT	TTTTAAGAGA	TGAAAGCAGA	4176
GTAAGTGGG	GGCTTAGGGA	ATCTGTAGGG	TTGCGGTATA	GCAATAGGGA	4226
GTTCTGGTCT	CTGAGAAGCA	GTCAGAGAGA	ATGGAAAACC	AGGCCCTTGC	4276
CAGTAGGTTA	GTGAGGTGGA	TATGATCAGA	TTATGGACAC	TCTCCAAATC	4326
ATAAATACTC	TAACAGCTAA	GGATCTCTGA	GGGAAACACA	ACAGGGAAAT	4376
ATTTTAGTTT	CTCCTTGAGA	AACAATGACA	AGACATAAAA	TTGGCAAGAA	4426
AGTCAGGAGT	GTATTCTAAT	AAGTGTGCT	TATCTCTTAT	TTTCTTCTAC	4476
AGTTGCAAAG	CCCAGAAGAA	AGAAATGGAC	AGCGGAAGAA	GTGGTTGTTT	4526
TTTTTTCCCC	TTCATTAATT	TTCTAGTTTT	TAGTAATCCA	GAAAATTTGA	4576
TTTTGTCTA	AAGTTCATTA	TGCAAAGATG	TCACCAACAG	ACTTCTGACT	4626
GCATGGTGAA	CTTTCATATG	ATACATAGGA	TTACACTTGT	ACCTGTTAAA	4676
AATAAAAGTT	TGACTTGCAT	AC			4698

- (2) INFORMATION FOR SEQUENCE ID NO: 6:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe
5

- (2) INFORMATION FOR SEQUENCE ID NO: 7:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2419 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	50
GGGGTCATCC	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCACCC	100
TCCTGGTAGC	ACTGAGAAGC	CAGGGCTGTG	CTTGCGGTCT	GCACCCTGAG	150
GGCCCGTGGA	TTCCTCTTCC	TGGAGCTCCA	GGAACCAGGC	AGTGAGGCCT	200
TGGTCTGAGA	CAGTATCCTC	AGGTCACAGA	GCAGAGGATG	CACAGGGTGT	250
GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	350
CCTGTAGAAT	CGACCTCTGC	TGGCCGGCTG	TACCCTGAGT	ACCCTCTCAC	400
TTCCTCCTTC	AGGTTTTTCAG	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	450
CTGGAGGCCA	CAGAGGAGCA	CCAAGGAGAA	GATCTGTAAG	TAGGCCTTTG	500
TTAGAGTCTC	CAAGGTTTCTC	TTCTCAGCTG	AGGCCTCTCA	CACACTCCCT	550
CTCTCCCCAG	GCCTGTGGGT	CTTCATTGCC	CAGCTCCTGC	CCACACTCCT	600
GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	650
ACTGCAAGCC	TGAGGAAGCC	CTTGAGGCCC	AACAAGAGGC	CCTGGGCCTG	700
GTGTGTGTGC	AGGCTGCCAC	CTCCTCCTCC	TCTCCTCTGG	TCCTGGGCAC	750
CCTGGAGGAG	GTGCCCACTG	CTGGGTCAAC	AGATCCTCCC	CAGAGTCCTC	800
AGGGAGCCTC	CGCCTTTCCC	ACTACCATCA	ACTTCACTCG	ACAGAGGCAA	850
CCCAGTGAGG	GTTCCAGCAG	CCGTGAAGAG	GAGGGGCCAA	GCACCTCTTG	900
TATCCTGGAG	TCCTTGTTCC	GAGCAGTAAT	CACATAAGAAG	GTGGCTGATT	950
TGGTTGGTTT	TCTGCTCCTC	AAATATCGAG	CCAGGGAGCC	AGTCACAAAG	1000
GCAGAAATGC	TGGAGAGTGT	CATCAAAAAT	TACAAGCACT	GTTTTCTCTGA	1050
GATCTTCGGC	AAAGCCTCTG	AGTCCTTGCA	GCTGGTCTTT	GGCATTGACG	1100
TGAAGGAAGC	AGACCCCACT	GGCCACTCCT	ATGTCCTTGT	CACCTGCCTA	1150
GGTCTCTCCT	ATGATGGCCT	GCTGGGTGAT	AATCAGATCA	TGCCCAAGAC	1200
AGGCTTCCTG	ATAATTGTCC	TGGTCATGAT	TGCAATGGAG	GGCGGCCATG	1250
CTCCTGAGGA	GGAAATCTGG	GAGGAGCTGA	GTGTGATGGA	GGTGTATGAT	1300
GGGAGGGAGC	ACAGTGCCTA	TGGGGAGCCC	AGGAAGCTGC	TCACCCAAGA	1350
TTTGGTGCAG	GAAAAGTACC	TGGAGTACGG	CAGGTGCCGG	ACAGTGATCC	1400

00019659.031797

CGCACGCTAT	GAGTTCCTGT	GGGGTCCAAG	GGCCCTCGCT	GAAACCAGCT	1450
ATGTGAAAGT	CCTTGAGTAT	GTGATCAAGG	TCAGTGCAAG	AGTTCGCTTT	1500
TTCTTCCCAT	CCCTGCGTGA	AGCAGCTTTG	AGAGAGGAGG	AAGAGGGAGT	1550
CTGAGCATGA	GTTGCAGCCA	AGGCCAGTGG	GAGGGGGACT	GGGCCAGTGC	1600
ACCTTCCAGG	GCCGCGTCCA	GCAGCTTCCC	CTGCCTCGTG	TGACATGAGG	1650
CCCATTCTTC	ACTCTGAAGA	GAGCGGTCAG	TGTTCTCAGT	AGTAGGTTTC	1700
TGTTCTATTG	GGTGACTTGG	AGATTTATCT	TTGTTCTCTT	TTGGAATTGT	1750
TCAAATGTTT	TTTTTTAAGG	GATGGTTGAA	TGAACTTCAG	CATCCAAGTT	1800
TATGAATGAC	AGCAGTCACA	CAGTTCTGTG	TATATAGTTT	AAGGGTAAGA	1850
GTCTTGTTG	TTATTTCAGAT	TGGGAAATCC	ATTCTATTTT	GTGAATTGGG	1900
ATAATAACAG	CAGTGGGAATA	AGTACTTAGA	AATGTGAAAA	ATGAGCAGTA	1950
AAATAGATGA	GATAAAGAAC	TAAAGAAATT	AAGAGATAGT	CAATTCTTGC	2000
CTTATACCTC	AGTCTATTCT	GTAAAATTTT	TAAAGATATA	TGCATACCTG	2050
GATTTCCCTG	GCTTCTTTGA	GAATGTAAGA	GAAATTAAAT	CTGAATAAAG	2100
AATTCCTTCT	GTTCACTGGC	TCTTTTCTTC	TCCATGCACT	GAGCATCTGC	2150
TTTTTGGAAG	GCCCTGGGTT	AGTAGTGGAG	ATGCTAAGGT	AAGCCAGACT	2200
CATACCCACC	CATAGGGTCG	TAGAGTCTAG	GAGCTGCAGT	CACGTAATCG	2250
AGGTGGCAAG	ATGTCCTCTA	AAGATGTAGG	GAAAAGTGAG	AGAGGGGTGA	2300
GGGTGTGGGG	CTCCGGGTGA	GAGTGGTGGA	GTGTCAATGC	CCTGAGCTGG	2350
GGCATTTTGG	GCTTTGGGAA	ACTGCAGTTC	CTTCTGGGGG	AGCTGATTGT	2400
AATGATCTTG	GGTGGATCC				2419

(2) INFORMATION FOR SEQUENCE ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5674 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-1 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCCGGGGCAC	CACTGGCATC	CCTCCCCCTA	CCACCCCCAA	TCCCTCCCTT	50
TACGCCACCC	ATCCAAACAT	CTTCACGCTC	ACCCCCAGCC	CAAGCCAGGC	100
AGAATCCGGT	TCCACCCCTG	CTCTCAACCC	AGGGAAGCCC	AGGTGCCCCG	150
ATGTGACGCC	ACTGACTTGA	GCATTAGTGG	TTAGAGAGAA	GCGAGGTTTT	200
CGGTCTGAGG	GGCGGCTTGA	GATCGGTGGA	GGGAAGCGGG	CCCAGCTCTG	250
TAAGGAGGCA	AGGTGACATG	CTGAGGGAGG	ACTGAGGACC	CACTTACCCC	300
AGATAGAGGA	CCCCAAATAA	TCCCTTCATG	CCAGTCCTGG	ACCATCTGGT	350
GGTGGACTTC	TCAGGCTGGG	CCACCCCCAG	CCCCCTTGCT	GCTTAAACCA	400
CTGGGGACTC	GAAGTCAGAG	CTCCGTGTGA	TCAGGGAAGG	GCTGCTTAGG	450
AGAGGGCAGC	GTCCAGGCTC	TGCCAGACAT	CATGCTCAGG	ATTCTCAAGG	500
AGGGCTGAGG	GTCCCTAAGA	CCCCACTCCC	GTGACCCAAC	CCCCACTCCA	550
ATGCTCACTC	CCGTGACCCA	ACCCCTCTTT	CATTGTCAAT	CCAACCCCCA	600
CCCCACATCC	CCCACCCCAT	CCCTCAACCC	TGATGCCCCAT	CCGCCCAGCC	650
ATTCCACCTT	CACCCCCACC	CCCACCCCCA	CGCCCACTCC	CACCCCCACC	700
CAGGCAGGAT	CCGGTTCCCG	CCAGGAAACA	TCCGGGTGCC	CGGATGTGAC	750
GCCACTGACT	TGCGCATTGT	GGGGCAGAGA	GAAGCGAGGT	TTCCATTCTG	800
AGGGACGGCG	TAGAGTTCGG	CCGAAGGAAC	CTGACCCAGG	CTCTGTGAGG	850
AGGCAAGGTG	AGAGGCTGAG	GGAGGACTGA	GGACCCCGCC	ACTCCAAATA	900

GAGAGCCCCA	AATATTCAG	CCCCGCCCTT	GCTGCCAGCC	CTGGCCCCACC	950
CGCGGGAAGA	CGTCTCAGCC	TGGGCTGCC	CCAGACCCTT	GCTCCAAAAG	1000
CCTTGAGAGA	CACCAGGTTT	TTCTCCCCAA	GCTCTGGAAT	CAGAGGTTGC	1050
TGTGACCAGG	GCAGGACTGG	TTAGGAGAGG	GCAGGGCACA	GGCTCTGCCA	1100
GGCATCAAGA	TCAGCACCCA	AGAGGGAGGG	CTGTGGGCCC	CCAAGACTGC	1150
ACTCCAATCC	CCACTCCCAC	CCCATTGCGA	TTCCCATTTCC	CCACCCAACC	1200
CCCATCTCCT	CAGCTACACC	TCCACCCCCA	TCCCTACTCC	TACTCCGTCA	1250
CCTGACCACC	ACCCTCCAGC	CCCAGCACCA	GCCCCAACCC	TTCTGCCACC	1300
TCACCCTCAC	TGCCCCCAAC	CCCACCCTCA	TCTCTCTCAT	GTGCCCCACT	1350
CCCATCGCCT	CCCCCATTCT	GGCAGAATCC	GGTTTGCCCC	TGCTCTCAAC	1400
CCAGGGAAGC	CCTGGTAGGC	CCGATGTGAA	ACCACTGACT	TGAACCTCAC	1450
AGATCTGAGA	GAAGCCAGGT	TCATTTAATG	GTTCTGAGGG	GCGGCTTGAG	1500
ATCCACTGAG	GGGAGTGTTT	TTAGGCTCTG	TGAGGAGGCA	AGGTGAGATG	1550
CTGAGGGAGG	ACTGAGGAGG	CACACACCCC	AGGTAGATGG	CCCCAAAATG	1600
ATCCAGTACC	ACCCCTGCTG	CCAGCCCTGG	ACCACCCGGC	CAGGACAGAT	1650
GTCTCAGCTG	GACCACCCCC	CGTCCCCTCC	CACTGCCACT	TAACCCACAG	1700
GGCAATCTGT	AGTCATAGCT	TATGTGACCG	GGGCAGGGTT	GGTCAGGAGA	1750
GGCAGGGCCC	AGGCATCAAG	GTCCAGCATC	CGCCCCGGCAT	TAGGGTCAGG	1800
ACCCTGGGAG	GGAAGTGAGG	GTTCCCCACC	CACACCTGTC	TCCTCATCTC	1850
CACCGCCACC	CCACTCACAT	TCCCATACTT	ACCCCTTACC	CCCAACCTCA	1900
TCTTGTCAGA	ATCCCTGCTG	TCAACCCACG	GAAGCCACGG	GAATGGCGGC	1950
CAGGCACTCG	GATCTTGACG	TCCCCATCCA	GGGTCTGATG	GAGGGAAGGG	2000
GCTTGAACAG	GGCCTCAGGG	GAGCAGAGGG	AGGGCCCTAC	TGCGAGATGA	2050
GGGAGGCCTC	AGAGGACCCA	GCACCCTAGG	ACACCGCACC	CCTGTCTGAG	2100
ACTGAGGCTG	CCACTTCTGG	CCTCAAGAAT	CAGAACGATG	GGGACTCAGA	2150
TTGCATGGGG	GTGGGACCCA	GGCCTGCAAG	GCTTACGCGG	AGGAAGAGGA	2200
GGGAGGACTC	AGGGGACCTT	GGAATCCAGA	TCAGTGTGGA	CCTCGGCCCT	2250
GAGAGGTCCA	GGGCACGGTG	GCCACATATG	GCCCATATTT	CCTGCATCTT	2300
TGAGGTGACA	GGACAGAGCT	GTGGTCTGAG	AAGTGGGGCC	TCAGGTCAAC	2350
AGAGGGAGGA	GTTCCAGGAT	CCATATGGCC	CAAGATGTGC	CCCCTTCATG	2400
AGGACTGGGG	ATATCCCCGG	CTCAGAAAGA	AGGGACTCCA	CACAGTCTGG	2450
CTGTCCCCTT	TTAGTAGCTC	TAGGGGGACC	AGATCAGGGA	TGGCGGTATG	2500
TTCCATTCTC	ACTTGTAACA	CAGGCAGGAA	GTTGGGGGGC	CCTCAGGGAG	2550
ATGGGGTCTT	GGGGTAAAGG	GGGGATGTCT	ACTCATGTCA	GGGAATTGGG	2600
GGTTGAGGAA	GCACAGGCGC	TGGCAGGAAT	AAAGATGAGT	GAGACAGACA	2650
AGGCTATTGG	AATCCACACC	CCAGAACCAA	AGGGGTGAGC	CCTGGACACC	2700
TCACCCAGGA	TGTGGCTTCT	TTTTCACTCC	TGTTTCCAGA	TCTGGGGCAG	2750
GTGAGGACCT	CATTCTCAGA	GGGTGACTCA	GGTCAACGTA	GGGACCCCCA	2800
TCTGGTCTAA	AGACAGAGCG	GTCCCAGGAT	CTGCCATGCG	TTCGGGTGAG	2850
GAACATGAGG	GAGGACTGAG	GGTACCCCAG	GACCAGAACA	CTGAGGGAGA	2900
CTGCACAGAA	ATCAGCCCTG	CCCCTGCTGT	CACCCCAGAG	AGCATGGGCT	2950
GGGCCGTCTG	CCGAGGTCCCT	TCCGTTATCC	TGGGATCATT	GATGTCAGGG	3000
ACGGGGAGGC	CTTGGTCTGA	GAAGGCTGCG	CTCAGGTCAG	TAGAGGGAGC	3050
GTCCCAGGGC	CTGCCAGTAG	TCAAGGTGAG	GACCAAGCGG	GCACCTCACC	3100
CAGGACACAT	TAATTCCAAT	GAATTTTGAT	ATCTCTTGCT	GCCCTTCCCC	3150
AAGACCTAG	GCACGTGTGG	CCAGATGTTT	GTCCCCCTCT	GTCCCTCCAT	3200
TCCTTATCAT	GGATGTGAAC	TCTGTATTG	GATTTCTCAG	ACCAGCAAAA	3250
GGGCAGGATC	CAGGCCCTGC	CAGGAAAAAT	ATAAGGGCCC	TGCGTGAGAA	3300
CAGAGGGGGT	CATCCACTGC	ATGAGAGTGG	GGATGTCACA	GAGTCCAGCC	3350
CACCCTCCTG	GTAGCACTGA	GAAGCCAGGG	CTGTGCTTGC	GGTCTGCACC	3400
CTGAGGGCCC	GTGGATTCCCT	CTTCCTGGAG	CTCCAGGAAC	CAGGCAGTGA	3450
GGCCTTGGTG	TGAGACAGTA	TCCTCAGGTC	ACAGAGCAGA	GGATGCACAG	3500
GGTGTGCCAG	CAGTGAATGT	TTGCCCTGAA	TGCACACCAA	GGGCCCCACC	3550
TGCCACAGGA	CACATAGGAC	TCCACAGAGT	CTGGCCTCAC	CTCCCTACTG	3600

TCAGTCCTGT	AGAATCGACC	TCTGCTGGCC	GGCTGTACCC	TGAGTACCCT	3650
CTCACTTCCT	CCTTCAGGTT	TTCAGGGGAC	AGGCCAACCC	AGAGGACAGG	3700
ATTCCTTGA	GGCCACAGAG	GAGCACCAAG	GAGAAGATCT	GTAAGTAGGC	3750
CTTTGTTAGA	GTCTCCAAGG	TTCAGTTCTC	AGCTGAGGCC	TCTCACACAC	3800
TCCCTCTCTC	CCCAGGCCTG	TGGGTCTTCA	TTGCCAGCT	CCTGCCCCACA	3850
CTCCTGCCTG	CTGCCCTGAC	GAGAGTCATC			3880
ATG TCT CTT	GAG CAG AGG	AGT CTG CAC	TGC AAG CCT	GAG GAA	3922
GCC CTT GAG	GCC CAA CAA	GAG GCC CTG	GGC CTG GTG	TGT GTG	3964
CAG GCT GCC	ACC TCC TCC	TCT TCT CTG	GTC CTG GGC	ACC	4006
CTG GAG GAG	GTG CCC ACT	GCT GGT TCA	ACA GAT CCT	CCC CAG	4048
AGT CCT CAG	GGG GCC TCC	GCC TTT CCC	ACT ACC ATC	AAC TTC	4090
ACT CGA CAG	AGG CAA CCC	AGT GAG GGT	TCC AGC AGC	CGT GAA	4132
GAG GAG GGG	CCA AGC ACC	TCT TGT ATC	CTG GAG TCC	TTG TTC	4174
CGA GCA GTA	ATC ACT AAG	AAG GTG GCT	GAT TTG GTT	GGT TTT	4216
CTG CTC CTC	AAA TAT CGA	GCC AGG GAG	CCA GTC ACA	AAG GCA	4258
GAA ATG CTG	GAG AGT GTC	ATC AAA AAT	TAC AAG CAC	TGT TTT	4300
CCT GAG ATC	TTC GGC AAA	GCC TCT GAG	TCC TTG CAG	CTG GTC	4342
TTT GGC ATT	GAC GTG AAG	GAA GCA GAC	CCC ACC GGC	CAC TCC	4384
TAT GTC CTT	GTC ACC TGC	CTA GGT CTC	TCC TAT GAT	GGC CTG	4426
CTG GGT GAT	AAT CAG ATC	ATG CCC AAG	ACA GGC TTC	CTG ATA	4468
ATT GTC CTG	GTC ATG ATT	GCA ATG GAG	GGC GGC CAT	GCT CCT	4510
GAG GAG GAA	ATC TGG GAG	GAG CTG AGT	GTG ATG GAG	GTG TAT	4552
GAT GGG AGG	GAG CAC AGT	GCC TAT GGG	GAG CCC AGG	AAG CTG	4594
CTC ACC CAA	GAT TTG GTG	CAG GAA AAG	TAC CTG GAG	TAC GGC	4636
AGG TGC CGG	ACA GTG ATC	CCG CAC GCT	ATG AGT TCC	TGT GGG	4678
GTC CAA GGG	CCC TCG CTG	AAA CCA GCT	ATG TGA		4711
AAGTCCTTGA	GTATGTGATC	AAGGTCAGTG	CAAGAGTTC		4750
GCTTTTTCTT	CCCATCCCTG	CGTGAAGCAG	CTTTGAGAGA	GGAGGAAGAG	4800
GGAGTCTGAG	CATGAGTTGC	AGCCAAGGCC	AGTGGGAGGG	GGACTGGGCC	4850
AGTGCACCTT	CCAGGGCCGC	GTCCAGCAGC	TTCCCCTGCC	TCGTGTGACA	4900
TGAGGCCCAT	TCTTCACTCT	GAAGAGAGCG	GTCAGTGTTT	TCAGTAGTAG	4950
GTTTCTGTTC	TATTGGGTGA	CTTGGAGATT	TATCTTTGTT	CTCTTTTGGA	5000
ATTGTTCAAA	TGTTTTTTTT	TAAGGGATGG	TTGAATGAAC	TTCAGCATCC	5050
AAGTTTATGA	ATGACAGCAG	TCACACAGTT	CTGTGTATAT	AGTTTAAGGG	5100
TAAGAGTCTT	GTGTTTTATT	CAGATTGGGA	AATCCATTCT	ATTTTGTGAA	5150
TTGGGATAAT	AACAGCAGTG	GAATAAGTAC	TTAGAAATGT	GAAAAATGAG	5200
CAGTAAAATA	GATGAGATAA	AGAACTAAAG	AAATTAAGAG	ATAGTCAATT	5250
CTTGCCTTAT	ACCTCAGTCT	ATTCTGTAAA	ATTTTAAAG	ATATATGCAT	5300
ACCTGGATTT	CCTTGGCTTC	TTTGAGAATG	TAAGAGAAAT	TAAATCTGAA	5350
TAAAGAATTC	TTCCTGTTCA	CTGGCTCTTT	TCTTCTCCAT	GCACTGAGCA	5400
TCTGCTTTTT	GGAAGGCCCT	GGGTAGTAG	TGGAGATGCT	AAGGTAAGCC	5450
AGACTCATAC	CCACCCATAG	GGTCGTAGAG	TCTAGGAGCT	GCAGTCACGT	5500
AATCGAGGTG	GCAAGATGTC	CTCTAAAGAT	GTAGGGAAAA	GTGAGAGAGG	5550
GGTGAGGGTG	TGGGGCTCCG	GGTGAGAGTG	GTGGAGTGTC	AATGCCCTGA	5600
GCTGGGGCAT	TTTGGGCTTT	GGGAAACTGC	AGTTCCTTCT	GGGGGAGCTG	5650
ATTGTAATGA	TCTTGGGTGG	ATCC			5674

(2) INFORMATION FOR SEQUENCE ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4157 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-2 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCCATCCAGA	TCCCCATCCG	GGCAGAATCC	GGTTCCACCC	TTGCCGTGAA	50
CCCAGGGAAG	TCACGGGCCC	GGATGTGACG	CCACTGACTT	GCACATTGGA	100
GGTCAGAGGA	CAGCGAGATT	CTCGCCCTGA	GCAACGGCCT	GACGTCGGCG	150
GAGGGAAGCA	GGCGCAGGCT	CCGTGAGGAG	GCAAGGTAAG	ACGCCGAGGG	200
AGGACTGAGG	CGGGCCTCAC	CCCAGACAGA	GGGCCCCCAA	TTAATCCAGC	250
GCTGCCTCTG	CTGCCGGGCC	TGGACCACCC	TGCAGGGGAA	GACTTCTCAG	300
GCTCAGTCGC	CACCACCTCA	CCCCGCCACC	CCCCGCCGCT	TTAACCGCAG	350
GGAActCTGG	CGTAAGAGCT	TTGTGTGACC	AGGGCAGGGC	TGGTTAGAAG	400
TGCTCAGGGC	CCAGACTCAG	CCAGGAATCA	AGGTCAGGAC	CCCAAGAGGG	450
GACTGAGGGC	AACCCACCCC	CTACCCTCAC	TACCAATCCC	ATCCCCCAAC	500
ACCAACCCCA	CCCCCATCCC	TCAAACACCA	ACCCACCCC	CAAACCCCAT	550
TCCCATCTCC	TCCCCCACCA	CCATCCTGGC	AGAATCCGGC	TTTGCCCCTG	600
CAATCAACCC	ACGGAAGCTC	CGGGAATGGC	GGCCAAGCAC	GCGGATCCTG	650
ACGTTACAT	GTACGGCTAA	GGGAGGGAAG	GGGTGGGTC	TCGTGAGTAT	700
GGCCTTTGGG	ATGCAGAGGA	AGGGCCCAGG	CCTCCTGGAA	GACAGTGGAG	750
TCCTTAGGGG	ACCCAGCATG	CCAGGACAGG	GGGCCCACTG	TACCCCTGTC	800
TCAAActGAG	CCACCTTTTC	ATTcAGCCGA	GGGAATCCTA	GGGATGCAGA	850
CCCACTTCAG	GGGGTTGGGG	CCCAGCCTGC	GAGGAGTCAA	GGGGAGGAAG	900
AAGAGGGAGG	ACTGAGGGGA	CCTTGAGATC	CAGATCAGTG	GCAACCTTGG	950
GCTGGGGGAT	CCTGGGCACA	GTGGCCGAAT	GTGCCCCGTG	CTCATTGCAC	1000
CTTCAGGGTG	ACAGAGAGTT	GAGGGCTGTG	GTCTGAGGGC	TGGGACTTCA	1050
GGTCAGCAGA	GGGAGGAATC	CCAGGATCTG	CCGGACCCAA	GGTGTGCCCC	1100
CTTCATGAGG	ACTCCCCATA	CCCCCGGCC	AGAAAGAAGG	GATGCCACAG	1150
AGTCTGGAAG	TAAATTGTTC	TTAGCTCTGG	GGGAACCTGA	TCAGGGATGG	1200
CCCTAAGTGA	CAATCTCATT	TGTACCACAG	GCAGGAGGTT	GGGGAACCTT	1250
CAGGGAGATA	AGGTGTTGGT	GTAAAGAGGA	GCTGTCTGCT	CATTTCAGGG	1300
GGTTCCCCCT	TGAGAAAGGG	CAGTCCCTGG	CAGGAGTAAA	GATGAGTAAC	1350
CCACAGGAGG	CCATCATAAC	GTTcACCCTA	GAACCAAAGG	GGTCAGCCCT	1400
GGACAACGCA	CGTGGGGTAA	CAGGATGTGG	CCCCTCCTCA	CTTGTCTTTC	1450
CAGATCTCAG	GGAGTTGATG	ACCTTGTTTT	CAGAAGGTGA	CTCAGTCAAC	1500
ACAGGGGCCC	CTCTGGTCGA	CAGATGCAGT	GGTTCTAGGA	TCTGCCAAGC	1550
ATCCAGGTGG	AGAGCCTGAG	GTAGGATTGA	GGGTACCCCT	GGGCCAGAAT	1600
GCAGCAAGGG	GGCCCCATAG	AAATCTGCCC	TGCCCCTGCG	GTTACTTCAG	1650
AGACCCTGGG	CAGGGCTGTC	AGCTGAAGTC	CCTCCATTAT	CTGGGATCTT	1700
TGATGTCAGG	GAAGGGGAGG	CCTTGGTCTG	AAGGGGCTGG	AGTCAGGTCA	1750
GTAGAGGGAG	GGTCTCAGGC	CCTGCCAGGA	GTGGACGTGA	GGACCAAGCG	1800
GACTCGTCAC	CCAGGACACC	TGGACTCCAA	TGAATTTGAC	ATCTCTCGTT	1850
GTCCTTCGCG	GAGGACCTGG	TCACGTATGG	CCAGATGTGG	GTCCCCTCTA	1900
TCTCCTTCTG	TACCATATCA	GGGATGTGAG	TTCTTGACAT	GAGAGATTCT	1950
CAAGCCAGCA	AAAGGGTGGG	ATTAGGCCCT	ACAAGGAGAA	AGGTGAGGGC	2000
CCTGAGTGAG	CACAGAGGGG	ACCCTCCACC	CAAGTAGAGT	GGGGACCTCA	2050

CCCTTCTGCTG

CGGAGTCTGG	CCAACCCCTGC	TGAGACTTCT	GGGAATCCGT	GGCTGTGCTT	2100
GCAGTCTGCA	CACTGAAGGC	CCGTGCATTC	CTCTCCCAGG	AATCAGGAGC	2150
TCCAGGAACC	AGGCAGTGAG	GCCTTGGTCT	GAGTCAGTGC	CTCAGGTCAC	2200
AGAGCAGAGG	GGACGCAGAC	AGTGCCAACA	CTGAAGGTTT	GCCTGGAATG	2250
CACACCAAGG	GCCCCACCCG	CCCAGAACAA	ATGGGACTCC	AGAGGGCCTG	2300
GCCTCACCCCT	CCCTATTCTC	AGTCCTGCAG	CCTGAGCATG	TGCTGGCCGG	2350
CTGTACCCTG	AGGTGCCCTC	CCACTTCCTC	CTTCAGGTTT	TGAGGGGGAC	2400
AGGCTGACAA	GTAGGACCCG	AGGCACTGGA	GGAGCATTGA	AGGAGAAGAT	2450
CTGTAAGTAA	GCCTTTGTCA	GAGCCTCCAA	GGTTCAGTTC	AGTTCTCACC	2500
TAAGGCCTCA	CACACGCTCC	TTCTCTCCCC	AGGCCTGTGG	GTCTTCATTG	2550
CCCAGCTCCT	GCCCGCACTC	CTGCCTGCTG	CCCTGACCAG	AGTCATC	2597
ATG CCT CTT	GAG CAG AGG	AGT CAG CAC	TGC AAG CCT	GAA GAA	2639
GGC CTT GAG	GCC CGA GGA	GAG GCC CTG	GGC CTG GTG	GGT GCG	2681
CAG GCT CCT	GCT ACT GAG	GAG CAG CAG	ACC GCT TCT	TCC TCT	2723
TCT ACT CTA	GTG GAA GTT	ACC CTG GGG	GAG GTG CCT	GCT GCC	2765
GAC TCA CCG	AGT CCT CCC	CAC AGT CCT	CAG GGA GCC	TCC AGC	2807
TTC TCG ACT	ACC ATC AAC	TAC ACT CTT	TGG AGA CAA	TCC GAT	2849
GAG GGC TCC	AGC AAC CAA	GAA GAG GAG	GGG CCA AGA	ATG TTT	2891
CCC GAC CTG	GAG TCC GAG	TTC CAA GCA	GCA ATC AGT	AGG AAG	2933
ATG GTT GAG	TTG GTT CAT	TTT CTG CTC	CTC AAG TAT	CGA GCC	2975
AGG GAG CCG	GTC ACA AAG	GCA GAA ATG	CTG GAG AGT	GTC CTC	3017
AGA AAT TGC	CAG GAC TTC	TTT CCC GTG	ATC TTC AGC	AAA GCC	3059
TCC GAG TAC	TTG CAG CTG	GTC TTT GGC	ATC GAG GTG	GTG GAA	3101
GTG GTC CCC	ATC AGC CAC	TTG TAC ATC	CTT GTC ACC	TGC CTG	3143
GGC CTC TCC	TAC GAT GGC	CTG CTG GGC	GAC AAT CAG	GTC ATG	3185
CCC AAG ACA	GGC CTC CTG	ATA ATC GTC	CTG GCC ATA	ATC GCA	3227
ATA GAG GGC	GAC TGT GCC	CCT GAG GAG	AAA ATC TGG	GAG GAG	3269
CTG AGT ATG	TTG GAG GTG	TTT GAG GGG	AGG GAG GAC	AGT GTC	3311
TTC GCA CAT	CCC AGG AAG	CTG CTC ATG	CAA GAT CTG	GTG CAG	3353
GAA AAC TAC	CTG GAG TAC	CGG CAG GTG	CCC GGC AGT	GAT CCT	3395
GCA TGC TAC	GAG TTC CTG	TGG GGT CCA	AGG GCC CTC	ATT GAA	3437
ACC AGC TAT	GTG AAA GTC	CTG CAC CAT	ACA CTA AAG	ATC GGT	3479
GGA GAA CCT	CAC ATT TCC	TAC CCA CCC	CTG CAT GAA	CGG GCT	3521
TTG AGA GAG	GGA GAA GAG	TGA			3542
GTCTCAGCAC	ATGTTGCAGC	CAGGGCCAGT	GGGAGGGGGT	CTGGGCCAGT	3592
GCACCTTCCA	GGGCCCCATC	CATTAGCTTC	CACTGCCTCG	TGTGATATGA	3642
GGCCCATTC	TGCCTCTTTG	AAGAGAGCAG	TCAGCATTCT	TAGCAGTGAG	3692
TTTCTGTTCT	GTTGGATGAC	TTTGAGATTT	ATCTTTCTTT	CCTGTTGGAA	3742
TTGTTCAAAT	GTTCTTTTAA	ACAAATGGTT	GGATGAACCT	CAGCATCCAA	3792
GTTTATGAAT	GACAGTAGTC	ACACATAGTG	CTGTTTATAT	AGTTTAGGGG	3842
TAAGAGTCCT	GTTTTTTTAT	CAGATTGGGA	AATCCATTCC	ATTTTGTGAG	3892
TTGTCACATA	ATAACAGCAG	TGGAATATGT	ATTTGCCTAT	ATTGTGAACG	3942
AATTAGCAGT	AAAATACATG	ATACAAGGAA	CTCAAAAGAT	AGTTAATTCT	3992
TGCCTTATAC	CTCAGTCTAT	TATGTAAAT	TAAAAATATG	TGTATGTTTT	4042
TGCTTCTTTG	AGAATGCAAA	AGAAATTAAA	TCTGAATAAA	TTCTTCCTGT	4092
TCAGTGGCTC	ATTTCTTTAC	CATTCACTCA	GCATCTGCTC	TGTGGAAGGC	4142
CCTGGTAGTA	GTGGG				4157

- (2) INFORMATION FOR SEQUENCE ID NO: 10:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 662 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-21 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATCCCCAT	GGATCCAGGA	AGAATCCAGT	TCCACCCCTG	CTGTGAACCC	50
AGGGAAGTCA	CGGGGCCGGA	TGTGACGCCA	CTGACTTGCG	CGTTGGAGGT	100
CAGAGAACAG	CGAGATTCTC	GCCCTGAGCA	ACGGCCTGAC	GTCGGCGGAG	150
GGAAGCAGGC	GCAGGCTCCG	TGAGGAGGCA	AGGTAAGATG	CCGAGGGAGG	200
ACTGAGGCGG	GCCTCACCCC	AGACAGAGGG	CCCCCAATAA	TCCAGCGCTG	250
CCTCTGCTGC	CAGGCCTGGA	CCACCCTGCA	GGGGAAGACT	TCTCAGGCTC	300
AGTCGCCACC	ACCTCACCCC	GCCACCCCCC	GCCGCTTTAA	CCGCAGGGAA	350
CTCTGGTGTA	AGAGCTTTGT	GTGACCAAGG	CAGGGCTGGT	TAGAAGTGCT	400
CAGGGCCCAG	ACTCAGCCAG	GAATCAAGGT	CAGGACCCCA	AGAGGGGACT	450
GAGGGTAACC	CCCCCGCACC	CCCACCACCA	TTCCCATCCC	CCAACACCAA	500
CCCCACCCCC	ATCCCCCAAC	ACCAAACCCA	CCACCATCGC	TCAAACATCA	550
ACGGCACCCC	CAAACCCCGA	TTCCCATCCC	CACCCATCCT	GGCAGAATCG	600
GAGCTTTGCC	CCTGCAATCA	ACCCACGGAA	GCTCCGGGAA	TGGCGGCCAA	650
GCACGCGGAT	CC				662

- (2) INFORMATION FOR SEQUENCE ID NO: 11:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1640 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cdna to mRNA
 - (ix) FEATURE:
 - (A) NAME/KEY: cdna MAGE-3
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GCCGCGAGGG	AAGCCGGCCC	AGGCTCGGTG	AGGAGGCAAG	GTTCTGAGGG	50
GACAGGCTGA	CCTGGAGGAC	CAGAGGCCCC	CGGAGGAGCA	CTGAAGGAGA	100
AGATCTGCCA	GTGGGTCTCC	ATTGCCCAGC	TCCTGCCCAC	ACTCCCGCCT	150
GTTGCCCTGA	CCAGAGTCAT	C			171
ATG CCT CTT	GAG CAG AGG	AGT CAG CAC	TGC AAG CCT	GAA GAA	213
GGC CTT GAG	GCC CGA GGA	GAG GCC CTG	GGC CTG GTG	GGT GCG	255
CAG GCT CCT	GCT ACT GAG	GAG CAG GAG	GCT GCC TCC	TCC TCT	297
TCT ACT CTA	GTT GAA GTC	ACC CTG GGG	GAG GTG CCT	GCT GCC	339
GAG TCA CCA	GAT CCT CCC	CAG AGT CCT	CAG GGA GCC	TCC AGC	381
CTC CCC ACT	ACC ATG AAC	TAC CCT CTC	TGG AGC CAA	TCC TAT	423
GAG GAC TCC	AGC AAC CAA	GAA GAG GAG	GGG CCA AGC	ACC TTC	465
CCT GAC CTG	GAG TCC GAG	TTC CAA GCA	GCA CTC AGT	AGG AAG	507

GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC	549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC	591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT	633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA	675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG	717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG	759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA	801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG	843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG	885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG	927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT	969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA	1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT	1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT	1095
TTG AGA GAG GGG GAA GAG TGA	1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT	1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA	1216
GGCCCATCTT TCACTCTTTG AAGCGAGCAG TCAGCATTTCT TAGTAGTGGG	1266
TTTCTGTTCT GTTGGATGAC TTTGAGATTA TTCTTTGTTT CCTGTTGGAG	1316
TTGTTCAAAT GTTCCTTTTA ACGGATGGTT GAATGAGCGT CAGCATCCAG	1366
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGAG	1416
TAAGAGTCTT GtTTTACT CAAATTgGGA AATCCATTCC ATTTTGTGAA	1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC	1516
GAATTAGCAA TAACATACAT GAGATACTC AAGAAATCAA AAGATAGTTG	1566
ATTCTTGCCT TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA	1616
ACCAGGATTT CCTTGACTTC TTTG	1640

(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 943 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-31 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA CCCAGTAGA GTGGGGACCT CACAGAGTCT GGCCAACCCT	50
CCTGACAGTT CTGGGAATCC GTGGCTGCGT TTGCTGTCTG CACATTGGGG	100
GCCCGTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG	150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGGCTCA	200
GATAGTGCCA ACGGTGAAGG TTTGCCTTGG ATTCAAACCA AGGGCCCCAC	250
CTGCCCCAGA ACACATGGAC TCCAGAGCGC CTGGCCTCAC CCTCAATACT	300
TTCAGTCCTG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC	350
CTCTCACTTC CTCCTTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC	400
AGAGGGCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAA GATAGCCTTTG	450
TTAGAGCCTC CAAGGTTCCTC TTCAGTACTC AGCTGAGGTC TCTCACATGC	500
TCCCTCTCTC CCCAGGCCAG TGGGTCTCCA TTGCCCAGCT CCTGCCCCACA	550
CTCCCCGCTG TTGCCCTGAC CAGAGTCATC	580

ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA	622
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG	664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT	706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC	748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC	790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT	832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC	874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG	916
GTG GCC AAG TTG GTT CAT TTT CTG CTC	943

(2) INFORMATION FOR SEQUENCE ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2531 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-4 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG	50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC	100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG	150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT	200
TGGTCTGAGA CAGTGTCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT	250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA	300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT	350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA	400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC	450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT	500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC	550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACCTCTG	600
CCTGCTGCCC TGACCAGAGT CATC	624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA	666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA	708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC	750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT	792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT	834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC	876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC	918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC	960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA	1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC	1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA	1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG	1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC	1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC	1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT	1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG	1296

00019559.02197

GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT	1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG	1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT	1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT	1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC	1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA	1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA	1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC	1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC	1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGT	1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT	1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT	1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG	1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG	1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT	1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA	2028
GTCTATTCTG TAAATTTTAA AAATATATAT GCATACCTGG ATTTCTTG	2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA	2128
ACTGGCTCAT TTCTTCTCTA TGCCTGAGC ATCTGCTCTG TGGAAGGCCC	2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA	2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT	2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT	2328
GAGAGTGGTC GGGTGTAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA	2378
AACTGCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC	2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC	2478
TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT	2528
GGG	2531

(2) INFORMATION FOR SEQUENCE ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2531 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-41 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG	50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC	100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG	150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT	200
TGGTCTGAGA CAGTGTCTCT AGGTTACAGA GCAGAGGATG CACAGGCTGT	250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA	300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT	350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA	400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC	450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT	500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC	550

TCTCCGTAGG	CCTGTGGGTC	CCCATTGCCC	AGCTTTTGCC	TGCACTCTTG	600
CCTGCTGCCC	TGAGCAGAGT	CATC			624
ATG TCT TCT	GAG CAG AAG	AGT CAG CAC	TGC AAG CCT	GAG GAA	666
GGC GTT GAG	GCC CAA GAA	GAG GCC CTG	GGC CTG GTG	GGT GCG	708
CAG GCT CCT	ACT ACT GAG	GAG CAG GAG	GCT GCT GTC	TCC TCC	750
TCC TCT CCT	CTG GTC CCT	GGC ACC CTG	GAG GAA GTG	CCT GCT	792
GCT GAG TCA	GCA GGT CCT	CCC CAG AGT	CCT CAG GGA	GCC TCT	834
GCC TTA CCC	ACT ACC ATC	AGC TTC ACT	TGC TGG AGG	CAA CCC	876
AAT GAG GGT	TCC AGC AGC	CAA GAA GAG	GAG GGG CCA	AGC ACC	918
TCG CCT GAC	GCA GAG TCC	TTG TTC CGA	GAA GCA CTC	AGT AAC	960
AAG GTG GAT	GAG TTG GCT	CAT TTT CTG	CTC CGC AAG	TAT CGA	1002
GCC AAG GAG	CTG GTC ACA	AAG GCA GAA	ATG CTG GAG	AGA GTC	1044
ATC AAA AAT	TAC AAG CGC	TGC TTT CCT	GTG ATC TTC	GGC AAA	1086
GCC TCC GAG	TCC CTG AAG	ATG ATC TTT	GGC ATT GAC	GTG AAG	1128
GAA GTG GAC	CCC ACC AGC	AAC ACC TAC	ACC CTT GTC	ACC TGC	1170
CTG GGC CTT	TCC TAT GAT	GGC CTG CTG	GGT AAT AAT	CAG ATC	1212
TTT CCC AAG	ACA GGC CTT	CTG ATA ATC	GTC CTG GGC	ACA ATT	1254
GCA ATG GAG	GGC GAC AGC	GCC TCT GAG	GAG GAA ATC	TGG GAG	1296
GAG CTG GGT	GTG ATG GGG	GTG TAT GAT	GGG AGG GAG	CAC ACT	1338
GTC TAT GGG	GAG CCC AGG	AAA CTG CTC	ACC CAA GAT	TGG GTG	1380
CAG GAA AAC	TAC CTG GAG	TAC CGG CAG	GTA CCC GGC	AGT AAT	1422
CCT GCG CGC	TAT GAG TTC	CTG TGG GGT	CCA AGG GCT	CTG GCT	1464
GAA ACC AGC	TAT GTG AAA	GTC CTG GAG	CAT GTG GTC	AGG GTC	1506
AAT GCA AGA	GTT CGC ATT	GCC TAC CCA	TCC CTG CGT	GAA GCA	1548
GCT TTG TTA	GAG GAG GAA	GAG GGA GTC	TGA		1578
GCATGAGTTG	CAGCCAGGGC	TGTGGGGAAG	GGGCAGGGCT	GGGCCAGTGC	1628
ATCTAACAGC	CCTGTGCAGC	AGCTTCCCTT	GCCTCGTGTA	ACATGAGGCC	1678
CATTCTTCAC	TCTGTTTGAA	GAAAATAGTC	AGTGTTCTTA	GTAGTGGGTT	1728
TCTATTTTGT	TGGATGACTT	GGAGATTTAT	CTCTGTTTCC	TTTTACAATT	1778
GTTGAAATGT	TCCTTTTAAT	GGATGGTTGA	ATTAACTTCA	GCATCCAAGT	1828
TTATGAATCG	TAGTTAACGT	ATATTGCTGT	TAATATAGTT	TAGGAGTAAG	1878
AGTCTTGTTT	TTTATTCAGA	TTGGGAAATC	CGTTCTATTT	TGTGAATTG	1928
GGACATAATA	ACAGCAGTGG	AGTAAGTATT	TAGAAGTGTG	AATTCACCGT	1978
GAAATAGGTG	AGATAAATTA	AAAGATACTT	AATTCCCGCC	TTATGCCTCA	2028
GTCTATTCTG	TAAAATTTAA	AAATATATAT	GCATACCTGG	ATTTCTTGG	2078
CTTCGTGAAT	GTAAGAGAAA	TTAAATCTGA	ATAAATAATT	CTTTCTGTTA	2128
ACTGGCTCAT	TTCTTCTCTA	TGCACTGAGC	ATCTGCTCTG	TGGAAGGCCC	2178
AGGATTAGTA	GTGGAGATAC	TAGGGTAAGC	CAGACACACA	CCTACCGATA	2228
GGGTATTAAG	AGTCTAGGAG	CGCGGTCATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAAGAT	GTAGGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAAT	TCCCTGTGTG	GGGCCTTTTG	GGCTTTGGGA	2378
AACTCCATTT	TCTTCTGAGG	GATCTGATTC	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGAAAA	GCCCAGATTG	GAAAAGTTGC	2478
TCTGAGCGGT	TCCTTTGTGA	CAATGGATGA	ACAGAGAGGA	GCCTCTACCT	2528
GGG					2531

(2) INFORMATION FOR SEQUENCE ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1068 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

- (A) NAME/KEY: cDNA MAGE-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

40
82
124
166
208
250
292
334
376
418
460
502
544
586
628
670
720
770
820
870
920
970
1020
1068

G GGG CCA AGC ACC TCG CCT GAC GCA GAG TCC TTG TTC CGA
GAA GCA CTC AGT AAC AAG GTG GAT GAG TTG GCT CAT TTT CTG
CTC CGC AAG TAT CGA GCC AAG GAG CTG GTC ACA AAG GCA GAA
ATG CTG GAG AGA GTC ATC AAA AAT TAC AAG CGC TGC TTT CCT
GTG ATC TTC GGC AAA GCC TCC GAG TCC CTG AAG ATG ATC TTT
GGC ATT GAC GTG AAG GAA GTG GAC CCC GCC AGC AAC ACC TAC
ACC CTT GTC ACC TGC CTG GGC CTT TCC TAT GAT GGC CTG CTG
GGT AAT AAT CAG ATC TTT CCC AAG ACA GGC CTT CTG ATA ATC
GTC CTG GGC ACA ATT GCA ATG GAG GGC GAC AGC GCC TCT GAG
GAG GAA ATC TGG GAG GAG CTG GGT GTG ATG GGG GTG TAT GAT
GGG AGG GAG CAC ACT GTC TAT GGG GAG CCC AGG AAA CTG CTC
ACC CAA GAT TGG GTG CAG GAA AAC TAC CTG GAG TAC CGG CAG
GTA CCC GGC AGT AAT CCT GCG CGC TAT GAG TTC CTG TGG GGT
CCA AGG GCT CTG GCT GAA ACC AGC TAT GTG AAA GTC CTG GAG
CAT GTG GTC AGG GTC AAT GCA AGA GTT CGC ATT GCC TAC CCA
TCC CTG CGT GAA GCA GCT TTG TTA GAG GAG GAA GAG GGA GTC
TGAGCATGAG TTGCAGCCAG GGCTGTGGGG AAGGGGCAGG GCTGGGCCAG
TGCATCTAAC AGCCCTGTGC AGCAGCTTCC CTTGCCTCGT GTAACATGAG
GCCCATTCTT CACTCTGTTT GAAGAAAATA GTCAGTGTTT TTAGTAGTGG
GTTTCTATTT TGTTGGATGA CTTGGAGATT TATCTCTGTT TCCTTTTACA
ATTGTTGAAA TGTTCTTTT AATGGATGGT TGAATTAAT TCAGCATCCA
AGTTTATGAA TCGTAGTTAA CGTATATTGC TGTTAATATA GTTTAGGAGT
AAGAGTCTTG TTTTATTATC AGATTGGGAA ATCCGTTCTA TTTTGTGAAT
TTGGGACATA ATAACAGCAG TGGAGTAAGT ATTTAGAAGT GTGAATTC

(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2226 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-5 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG 50
GGGACCATTG ACCCAAGAG GGTGGAGACC TCACAGATTG CAGCCTACCC 100

TCCTGTTAGC	ACTGGGGGCC	TGAGGCTGTG	CTTGCAGTCT	GCACCCTGAG	150
GGCCCATGCA	TTCCTCTTCC	AGGAGCTCCA	GGAAACAGAC	ACTGAGGCCT	200
TGGTCTGAGG	CCGTGCCCTC	AGGTCACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCCAGC	AGTGAACGTT	TGCCTTGAAT	GCACACTAAT	GGCCCCCATC	300
GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCT	GAGGTGCCCT	400
CTCACTTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCAG	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTTGT	TAGAGCCTCC	AAGGTTTCAGT	TTTTAGCTGA	550
GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGTC	TCCATTGCCC	600
AGCTCCTGCC	CACACTCCTG	CCTGTTGCGG	TGACCAGAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG	AGT CAG CAC	TGC AAG CCT	GAG GAA	686
CTC CTC TGG	TCC CAG GCA	CCC TGG GGG	AGG TGC CTG	CTG CTG CTG	728
GGT CAC CAG	GTC CTC TCA	AGA GTC CTC	AGG GAG CCT	CCG CCA	770
TCC CCA CTG	CCA TCG ATT	TCA CTC TAT	GGA GGC AAT	CCA TTA	812
AGG GCT CCA	GCA ACC AAG	AAG AGG AGG	GGC CAA GCA	CCT CCC	854
CTG ACC CAG	AGT CTG TGT	TCC GAG CAG	CAC TCA GTA	AGA AGG	896
TGG CTG ACT	TGA				908
TTCATTTTCT	GCTCCTCAAG	TATTAAGTCA	AGGAGCTGGT	CACAAAGGCA	958
GAAATGCTGG	AGAGCGTCAT	CAAAAATTAC	AAGCGCTGCT	TTCCTGAGAT	1008
CTTCGGCAAA	GCCTCCGAGT	CCTTGCAGCT	GGTCTTTGGC	ATTGACGTGA	1058
AGGAAGCGGA	CCCCACCAGC	AACACCTACA	CCCTTGTCAC	CTGCCTGGGA	1108
CTCCTATGAT	GGCCTGCTGG	TTGATAATAA	TCAGATCATG	CCCAAGACGG	1158
GCCTCCTGAT	AATCGTCTTG	GGCATGATTG	CAATGGAGGG	CAAATGCGTC	1208
CCTGAGGAGA	AAATCTGGGA	GGAGCTGAGT	GTGATGAAGG	TGTATGTTGG	1258
GAGGGAGCAC	AGTGTCTGTG	GGGAGCCCAG	GAAGCTGCTC	ACCCAAGATT	1308
TGGTGCAGGA	AAACTACCTG	GAGTACCGGC	AGGTGCCCAG	CAGTGATCCC	1358
ATATGCTATG	AGTTACTGTG	GGGTCCAAGG	GCACTCGCTG	CTTGAAAGTA	1408
CTGGAGCACG	TGGTCAGGGT	CAATGCAAGA	GTTCTCATTT	CCTACCCATC	1458
CCTGCGTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1508
CTGCAGCCAG	GGCCACTGCG	AGGGGGGCTG	GGCCAGTGCA	CCTTCCAGGG	1558
CTCCGTCCAG	TAGTTTCCCC	TGCCTTAATG	TGACATGAGG	CCCATTCTTC	1608
TCTCTTTGAA	GAGAGCAGTC	AACATTCTTA	GTAGTGGGTT	TCTGTTCTAT	1658
TGGATGACTT	TGAGATTTGT	CTTTGTTTCC	TTTTGGAATT	GTTCAAATGT	1708
TTCTTTTAAAT	GGGTGGTTGA	ATGAACTTCA	GCATTCAAAT	TTATGAATGA	1758
CAGTAGTCAC	ACATAGTGCT	GTTTATATAG	TTTAGGAGTA	AGAGTCTTGT	1808
TTTTTTATTCA	GATTGGGAAA	TCCATTCCAT	TTTGTGAATT	GGGACATAGT	1858
TACAGCAGTG	GAATAAGTAT	TCATTTAGAA	ATGTGAATGA	GCAGTAAAAC	1908
TGATGACATA	AAGAAATTAA	AAGATATTTA	ATTCTTGCTT	ATACTCAGTC	1958
TATTCGGTAA	AATTTTTTTT	AAAAAATGTG	CATACCTGGA	TTTCCTTGGC	2008
TTCTTTTGAGA	ATGTAAGACA	AATTAAATCT	GAATAAATCA	TTCTCCCTGT	2058
TCACTGGCTC	ATTTATTCTC	TATGCACTGA	GCATTTGCTC	TGTGGAAGGC	2108
CCTGGGGTTAA	TAGTGGAGAT	GCTAAGGTAA	GCCAGACTCA	CCCCTACCCA	2158
CAGGGTAGTA	AAGTCTAGGA	GCAGCAGTCA	TATAATTAAG	GTGGAGAGAT	2208
GCCCTCTAAG	ATGTAGAG				2226

- (2) INFORMATION FOR SEQUENCE ID NO: 17:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2305 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-51 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATCCAGGC	CTTGCCAGGA	GAAAGGTGAG	GGCCCTGTGT	GAGCACAGAG	50
GGGACCATTTC	ACCCCAAGAG	GGTGGAGACC	TCACAGATTTC	CAGCCTACCC	100
TCCTGTTAGC	ACTGGGGGCC	TGAGGCTGTG	CTTGCAGTCT	GCACCCTGAG	150
GGCCCATGCA	TTCCTCTTCC	AGGAGCTCCA	GGAAACAGAC	ACTGAGGCCT	200
TGGTCTGAGG	CCGTGCCCTC	AGGTCACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCCAGC	AGTGAACGTT	TGCCTTGAAT	GCACACTAAT	GGCCCCCATC	300
GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCT	GAGGTGCCCT	400
CTCACTTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCAG	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTTGT	TAGAGCCTCC	AAGGTTCACT	TTTGTAGCTGA	550
GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGTC	TCCATTGCCC	600
AGCTCCTGCC	CACACTCCTG	CCTGTTGCGG	TGACCAGAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG	AGT CAG CAC	TGC AAG CCT	GAG GAA	686
GGC CTT GAC	ACC CAA GAA	GAG CCC TGG	GCC TGG TGG	GTG TGC	728
AGG CTG CCA	CTA CTG AGG	AGC AGG AGG	CTG TGT CCT	CCT CCT	770
CTC CTC TGG	TCC CAG GCA	CCC TGG GGG	AGG TGC CTG	CTG CTG	812
GGT CAC CAG	GTC CTC TCA	AGA GTC CTC	AGG GAG CCT	CCG CCA	854
TCC CCA CTG	CCA TCG ATT	TCA CTC TAT	GGA GGC AAT	CCA TTA	896
AGG GCT CCA	GCA ACC AAG	AAG AGG AGG	GGC CAA GCA	CCT CCC	938
CTG ACC CAG	AGT CTG TGT	TCC GAG CAG	CAC TCA GTA	AGA AGG	980
TGG CTG ACT	TGA				992
TTCATTTTCT	GCTCCTCAAG	TATTAAGTCA	AGGAGCCGGT	CACAAAGGCA	1042
GAAATGCTGG	AGAGCGTCAT	CAAAAATTAC	AAGCGCTGCT	TTCCTGAGAT	1092
CTTCGGCAAA	GCCTCCGAGT	CCTTGACAGT	GGTCTTTGGC	ATTGACGTGA	1142
AGGAAGCGGA	CCCCACCAGC	AACACCTACA	CCCTTGTCAC	CTGCCTGGGA	1192
CTCCTATGAT	GGCCTGGTGG	TTTAATCAGA	TCATGCCCAA	GACGGGCCTC	1242
CTGATAATCG	TCTTGGGCAT	GATTGCAATG	GAGGGCAAAT	GCGTCCCTGA	1292
GGAGAAAATC	TGGGAGGAGC	TGGGTGTGAT	GAAGGTGTAT	GTTGGGAGGG	1342
AGCACAGTGT	CTGTGGGGAG	CCCAGGAAGC	TGCTCACCCA	AGATTTGGTG	1392
CAGGAAAAC	ACCTGGAGTA	CCGCAGGTGC	CCAGCAGTGA	TCCCATATGC	1442
TATGAGTTAC	TGTGGGGTCC	AAGGGCACTC	GCTGCTTGAA	AGTACTGGAG	1492
CACGTGGTCA	GGGTCAATGC	AAGAGTTCTC	ATTTCTTACC	CATCCCTGCA	1542
TGAAGCAGCT	TTGAGAGAGG	AGGAAGAGGG	AGTCTGAGCA	TGAGCTGCAG	1592
CCAGGGCCAC	TGCGAGGGGG	GCTGGGCCAG	TGCACCTTCC	AGGGCTCCGT	1642
CCAGTAGTTT	CCCCTGCCTT	AATGTGACAT	GAGGCCCAT	CTTCTCTCTT	1692
TGAAGAGAGC	AGTCAACATT	CTTAGTAGTG	GGTTTCTGTT	CTATTGGATG	1742
ACTTTGAGAT	TTGTCTTTGT	TTCCTTTTGG	AATTGTTCAA	ATGTTCTTTT	1792
TAATGGGTGG	TTGAATGAAC	TTCAGCATT	AAATTTATGA	ATGACAGTAG	1842
TCACACATAG	TGCTGTTTAT	ATAGTTTAGG	AGTAAGAGTC	TTGTTTTTTA	1892
TTCAGATTGG	GAAATCCATT	CCATTTTGTG	AATTGGGACA	TAGTTACAGC	1942

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AGTGAATATA	GTATTCATTT	AGAAATGTGA	ATGAGCAGTA	AAACTGATGA	1992
GATAAAGAAA	TTAAAAGATA	TTTAATTCTT	GCCTTATACT	CAGTCTATTC	2042
GGTAAAATTT	TTTTTTTAAA	ATGTGCATAC	CTGGATTTCC	TTGGCTTCTT	2092
TGAGAATGTA	AGACAAATTA	AATCTGAATA	AATCATTCTC	CCTGTTCACT	2142
GGCTCATTTA	TTCTCTATGC	ACTGAGCATT	TGCTCTGTGG	AAGGCCCTGG	2192
GTTAATAGTG	GAGATGCTAA	GGTAAGCCAG	ACTCACCCCT	ACCCACAGGG	2242
TAGTAAAGTC	TAGGAGCAGC	AGTCATATAA	TTAAGGTGGA	GAGATGCCCT	2292
CTAAGATGTA	GAG				2305

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 225 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-6 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TAT	TTC	TTT	CCT	GTG	ATC	TTC	AGC	AAA	GCT	TCC	GAT	TCC	TTG	42
CAG	CTG	GTC	TTT	GGC	ATC	GAG	CTG	ATG	GAA	GTG	GAC	CCC	ATC	84
GGC	CAC	GTG	TAC	ATC	TTT	GCC	ACC	TGC	CTG	GGC	CTC	TCC	TAC	126
GAT	GGC	CTG	CTG	GGT	GAC	AAT	CAG	ATC	ATG	CCC	AGG	ACA	GGC	168
TTC	CTG	ATA	ATC	ATC	CTG	GCC	ATA	ATC	GCA	AGA	GAG	GGC	GAC	210
TGT	GCC	CCT	GAG	GAG										225

(2) INFORMATION FOR SEQUENCE ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1947 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-7 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA	ACAAGGGCCC	CACACTCCCC	AGAACACAAG	GGAATCCAGA	50
GAGCCCAGCC	TCACCTTCCC	TACTGTCACT	CCTGCAGCCT	CAGCCTCTGC	100
TGGCCGGCTG	TACCCTGAGG	TGCCCTCTCA	CTTCCTCCTT	CAGGTTCTCA	150
GCGGACAGGC	CGGCCAGGAG	GTCAGAAGCC	CCAGGAGGCC	CCAGAGGAGC	200
ACCGAAGGAG	AAGATCTGTA	AGTAGGCCTT	TGTTAGGGCC	TCCAGGGCGT	250
GGTTCACAAA	TGAGGCCCTT	CACAAGCTCC	TTCTCTCCCC	AGATCTGTGG	300
GTCCTCCCC	ATCGCCCAGC	TGCTGCCCCG	ACTCCAGCCT	GCTGCCCTGA	350
CCAGAGTCAT	CATGTCTTCT	GAGCAGAGGA	GTCAGCACTG	CAAGCCTGAG	400

GATGCCTTGA	GGCCCAAGGA	CAGGAGGCTC	TGGGCCTGGT	GGGTGCGCAG	450
GCTCCCGCCA	CCGAGGAGCA	CGAGGCTGCC	TCCTCCTTCA	CTCTGATTGA	500
AGGCACCTTG	GAGGAGGTGC	CTGCTGCTGG	GTCCCCCAGT	CCTCCCCTGA	550
GTCTCAGGGT	TCCTCCTTTT	CCCTGACCAT	CAGCAACAAC	ACTCTATGGA	600
GCCAATCCAG	TGAGGGCACC	AGCAGCCGGG	AAGAGGAGGG	GCCAACCACC	650
TAGACACACC	CCGCTCACCT	GGCGTCCTTG	TTCCA		685
ATG GGA AGG	TGG CTG AGT	TGG TTC GCT	TCC TGC TGC	ACA AGT	727
ATC GAG TCA	AGG AGC TGG	TCA CAA AGG	CAG AAA TGC	TGG ACA	769
GTG TCA TCA	AAA ATT ACA	AGC ACT AGT	TTC CTT GTG	ATC TAT	811
GGC AAA GCC	TCA GAG TGC	ATG CAG GTG	ATG TTT GGC	ATT GAC	853
ATG AAG GAA	GTG GAC CCC	GCG GCC ACT	CCT ACG TCC	TTG TCA	895
CCT GCT TGG	GCC TCT CCT	ACA ATG GCC	TGC TGG GTG	ATG ATC	937
AGA GCA TGC	CCG AGA CCG	GCC TTC TGA			964
TTATGGTCTT	GACCATGATC	TTAATGGAGG	GCCACTGTGC	CCCTGAGGAG	1014
GCAATCTGGG	AAGCGTTGAG	TGTAATGGTG	TATGATGGGA	TGGAGCAGTT	1064
TCTTTGGGCA	GCTGAGGAAG	CTGCTCACCC	AAGATTGGGT	GCAGGAAAAC	1114
TACCTGCAAT	ACCGCCAGGT	GCCCAGCAGT	GATCCCCCGT	GCTACCAGTT	1164
CCTGTGGGGT	CCAAGGGCCC	TCATTGAAAC	CAGCTATGTG	AAAGTCCTGG	1214
AGTATGCAGC	CAGGGTCAGT	ACTAAAGAGA	GCATTTCTTA	CCCATCCCTG	1264
CATGAAGAGG	CTTTGGGAGA	GGAGGAAGAG	GGAGTCTGAG	CAGAAGTTGC	1314
AGCCAGGGCC	AGTGGGGCAG	ATTGGGGGAG	GGCCTGGGCA	GTGCACGTTC	1364
CACACATCCA	CCACCTTCCC	TGTCCTGTTA	CATGAGGCCC	ATTCTTCACT	1414
CTGTGTTTGA	AGAGAGCAGT	CAATGTTCTC	AGTAGCGGGG	AGTGTGTTGG	1464
GTGTGAGGGA	ATACAAGGTG	GACCATCTCT	CAGTTCCTGT	TCTCTTGGGC	1514
GATTTGGAGG	TTTATCTTTG	TTTCCTTTTG	CAGTCGTTCA	AATGTTCCCT	1564
TTAATGGATG	GTGTAATGAA	CTTCAACATT	CATTTTCATGT	ATGACAGTAG	1614
GCAGACTTAC	TGTTTTTTAT	ATAGTTAAAA	GTAAGTGCAT	TGTTTTTTAT	1664
TTATGTAAGA	AAATCTATGT	TATTTCTTGA	ATTGGGACAA	CATAACATAG	1714
CAGAGGATTA	AGTACCTTTT	ATAATGTGAA	AGAACAAAGC	GGTAAAATGG	1764
GTGAGATAAA	GAAATAAAGA	AATTAAATTG	GCTGGGCACG	GTGGCTCACG	1814
CCTGTAATCC	CAGCACTTTA	GGAGGCAGAG	GCACGGGGAT	CACGAGGTCA	1864
GGAGATCGAG	ACCATTCTGG	CTAACACAGT	GAAACACCAT	CTCTATTAAA	1914
AATACAAAAC	TTAGCCGGGC	GTGGTGGCGG	GTG		1947

(2) INFORMATION FOR SEQUENCE ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1810 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-8 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GAGCTCCAGG	AACCAGGCTG	TGAGGTCTTG	GTCTGAGGCA	GTATCTTCAA	50
TCACAGAGCA	TAAGAGGCCC	AGGCAGTAGT	AGCAGTCAAG	CTGAGGTGGT	100
GTTTCCCCTG	TATGTATACC	AGAGGCCCTT	CTGGCATCAG	AACAGCAGGA	150
ACCCACAGT	TCCTGGCCCT	ACCAGCCCTT	TTGTCAGTCC	TGGAGCCTTG	200
GCCTTTGCCA	GGAGGCTGCA	CCCTGAGATG	CCCTCTCAAT	TTCTCCTTCA	250
GGTTCGCAGA	GAACAGGCCA	GCCAGGAGGT	CAGGAGGCCC	CAGAGAAGCA	300

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CTGAAGAAGA	CCTGTAAGTA	GACCTTTGTT	AGGGCATCCA	GGGTGTAGTA	350
CCCAGCTGAG	GCCTCTCACA	CGCTTCCTCT	CTCCCCAGGC	CTGTGGGTCT	400
CAATTGCCCA	GCTCCGGCCC	ACACTCTCCT	GCTGCCCTGA	CCTGAGTCAT	450
C					451
ATG CTT CTT	GGG CAG AAG	AGT CAG CGC	TAC AAG GCT	GAG GAA	493
GGC CTT CAG	GCC CAA GGA	GAG GCA CCA	GGG CTT ATG	GAT GTG	535
CAG ATT CCC	ACA GCT GAG	GAG CAG AAG	GCT GCA TCC	TCC TCC	577
TCT ACT CTG	ATC ATG GGA	ACC CTT GAG	GAG GTG ACT	GAT TCT	619
GGG TCA CCA	AGT CCT CCC	CAG AGT CCT	GAG GGT GCC	TCC TCT	661
TCC CTG ACT	GTC ACC GAC	AGC ACT CTG	TGG AGC CAA	TCC GAT	703
GAG GGT TCC	AGC AGC AAT	GAA GAG GAG	GGG CCA AGC	ACC TCC	745
CCG GAC CCA	GCT CAC CTG	GAG TCC CTG	TTC CGG GAA	GCA CTT	787
GAT GAG AAA	GTG GCT GAG	TTA GTT CGT	TTC CTG CTC	CGC AAA	829
TAT CAA ATT	AAG GAG CCG	GTC ACA AAG	GCA GAA ATG	CTT GAG	871
AGT GTC ATC	AAA AAT TAC	AAG AAC CAC	TTT CCT GAT	ATC TTC	913
AGC AAA GCC	TCT GAG TGC	ATG CAG GTG	ATC TTT GGC	ATT GAT	955
GTG AAG GAA	GTG GAC CCT	GCC GGC CAC	TCC TAC ATC	CTT GTC	997
ACC TGC CTG	GGC CTC TCC	TAT GAT GGC	CTG CTG GGT	GAT GAT	1039
CAG AGT ACG	CCC AAG ACC	GGC CTC CTG	ATA ATC GTC	CTG GGC	1081
ATG ATC TTA	ATG GAG GGC	AGC CGC GCC	CCG GAG GAG	GCA ATC	1123
TGG GAA GCA	TTG AGT GTG	ATG GGG GCT	GTA TGA		1156
TGGGAGGGAG	CACAGTGTCT	ATTGGAAGCT	CAGGAAGCTG	CTCACCCAAG	1206
AGTGGGTGCA	GGAGAACTAC	CTGGAGTACC	GCCAGGCGCC	CGGCAGTGAT	1256
CCTGTGCGCT	ACGAGTTCCT	GTGGGGTCCA	AGGGCCCTTG	CTGAAACCAG	1306
CTATGTGAAA	GTCCTGGAGC	ATGTGGTCAG	GGTCAATGCA	AGAGTTCGCA	1356
TTTCCTACCC	ATCCCTGCAT	GAAGAGGCTT	TGGGAGAGGA	GAAAGGAGTT	1406
TGAGCAGGAG	TTGCAGCTAG	GGCCAGTGGG	GCAGGTTGTG	GGAGGGCCTG	1456
GGCCAGTGCA	CGTTCCAGGG	CCACATCCAC	CACTTTCCCT	GCTCTGTTAC	1506
ATGAGGCCCA	TTCTTCACTC	TGTGTTTGAA	GAGAGCAGTC	ACAGTTCTCA	1556
GTAGTGGGGA	GCATGTTGGG	TGTGAGGGAA	CACAGTGTGG	ACCATCTCTC	1606
AGTTCCTGTT	CTATTGGGCG	ATTTGGAGGT	TTATCTTTGT	TTCCTTTTGG	1656
AATTGTTCCA	ATGTTCTTTC	TAATGGATGG	TGTAATGAAC	TTCAACATTC	1706
ATTTTATGTA	TGACAGTAGA	CAGACTTACT	GCTTTTTATA	TAGTTTAGGA	1756
GTAAGAGTCT	TGCTTTTCAT	TTATACTGGG	AAACCCATGT	TATTTCTTGA	1806
ATTC					1810

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1412 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-9 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TCTGAGACAG	TGTCCTCAGG	TCGCAGAGCA	GAGGAGACCC	AGGCAGTGTC	50
AGCAGTGAAG	GTGAAGTGTT	CACCCTGAAT	GTGCACCAAG	GGCCCCACCT	100
GCCCCAGCAC	ACATGGGACC	CCATAGCACC	TGGCCCCATT	CCCCCTACTG	150
TCACTCATAG	AGCCTTGATC	TCTGCAGGCT	AGCTGCACGC	TGAGTAGCCC	200

TCTCACTTCC	TCCCTCAGGT	TCTCGGGACA	GGCTAACCAG	GAGGACAGGA	250
GCCCCAAGAG	GCCCCAGAGC	AGCATTGACG	AAGACCTGTA	AGTCAGCCTT	300
TGTTAGAACC	TCCAAGGTTT	GGTTCTCAGC	TGAAGTCTCT	CACACACTCC	350
CTCTCTCCCC	AGGCCTGTGG	GTCTCCATCG	CCCAGCTCCT	GCCCACGCTC	400
CTGACTGCTG	CCCTGACCAG	AGTCATC			427
ATG TCT CTC	GAG CAG	AGG AGT CCG	CAC TGC AAG	CCT GAT GAA	469
GAC CTT GAA	GCC CAA	GGA GAG GAC	TTG GGC CTG	ATG GGT GCA	511
CAG GAA CCC	ACA GGC	GAG GAG GAG	GAG ACT ACC	TCC TCC TCT	553
GAC AGC AAG	GAG GAG	GAG GTG TCT	GCT GCT GGG	TCA TCA AGT	595
CCT CCC CAG	AGT CCT	CAG GGA GGC	GCT TCC TCC	TCC ATT TCC	637
GTC TAC TAC	ACT TTA	TGG AGC CAA	TTC GAT GAG	GGC TCC AGC	679
AGT CAA GAA	GAG GAA	GAG CCA AGC	TCC TCG GTC	GAC CCA GCT	721
CAG CTG GAG	TTC ATG	TTC CAA GAA	GCA CTG AAA	TTG AAG GTG	763
GCT GAG TTG	GTT CAT	TTC CTG CTC	CAC AAA TAT	CGA GTC AAG	805
GAG CCG GTC	ACA AAG	GCA GAA ATG	CTG GAG AGC	GTC ATC AAA	847
AAT TAC AAG	CGC TAC	TTT CCT GTG	ATC TTC GGC	AAA GCC TCC	889
GAG TTC ATG	CAG GTG	ATC TTT GGC	ACT GAT GTG	AAG GAG GTG	931
GAC CCC GCC	GGC CAC	TCC TAC ATC	CTT GTC ACT	GCT CTT GGC	973
CTC TCG TGC	GAT AGC	ATG CTG GGT	GAT GGT CAT	AGC ATG CCC	1015
AAG GCC GCC	CTC CTG	ATC ATT GTC	CTG GGT GTG	ATC CTA ACC	1057
AAA GAC AAC	TGC GCC	CCT GAA GAG	GTT ATC TGG	GAA GCG TTG	1099
AGT GTG ATG	GGG GTG	TAT GTT GGG	AAG GAG CAC	ATG TTC TAC	1141
GGG GAG CCC	AGG AAG	CTG CTC ACC	CAA GAT TGG	GTG CAG GAA	1183
AAC TAC CTG	GAG TAC	CGG CAG GTG	CCC GGC AGT	GAT CCT GCG	1225
CAC TAC GAG	TTC CTG	TGG GGT TCC	AAG GCC CAC	GCT GAA ACC	1267
AGC TAT GAG	AAG GTC	ATA AAT TAT	TTG GTC ATG	CTC AAT GCA	1309
AGA GAG CCC	ATC TGC	TAC CCA TCC	CTT TAT GAA	GAG GTT TTG	1351
GGA GAG GAG	CAA GAG	GGA GTC TGA			1375
GCACCAGCCG	CAGCCGGGGC	CAAAGTTTGT	GGGGTCA		1412

ACCTGCTCCA	GGACAAAGTG	GACCCCACTG	CATCAGCTCC	ACCTACCCTA	50
CTGTCACTCC	TGGAGCCTTG	GCCTCTGCCG	GCTGCATCCT	GAGGAGCCAT	100
CTCTCACTTC	CTTCTTCAGG	TTCTCAGGGG	ACAGGGAGAG	CAAGAGGTCA	150
AGAGCTGTGG	GACACCACAG	AGCAGCACTG	AAGGAGAAGA	CCTGTAAGTT	200
GGCCTTTGTT	AGAACCTCCA	GGGTGTGGTT	CTCAGCTGTG	GCCACTTACA	250
CCCTCCCTCT	CTCCCCAGGC	CTGTGGGTCC	CCATCGCCCA	AGTCCTGCCC	300
ACACTCCCAC	CTGCTACCCT	GATCAGAGTC	ATC		333
ATG CCT CGA	GCT CCA AAG	CGT CAG CGC	TGC ATG CCT	GAA GAA	375
GAT CTT CAA	TCC CAA AGT	GAG ACA CAG	GGC CTC GAG	GGT GCA	417
CAG GCT CCC	CTG GCT GTG	GAG GAG GAT	GCT TCA TCA	TCC ACT	459

TCC ACC AGC TCC TCT TTT CCA TCC TCT TTT CCC TCC TCC TCC	501
TCT TCC TCC TCC TCC TCC TGC TAT CCT CTA ATA CCA AGC ACC	543
CCA GAG GAG GTT TCT GCT GAT GAT GAG ACA CCA AAT CCT CCC	585
CAG AGT GCT CAG ATA GCC TGC TCC TCC CCC TCG GTC GTT GCT	627
TCC CTT CCA TTA GAT CAA TCT GAT GAG GGC TCC AGC AGC CAA	669
AAG GAG GAG AGT CCA AGC ACC CTA CAG GTC CTG CCA GAC AGT	711
GAG TCT TTA CCC AGA AGT GAG ATA GAT GAA AAG GTG ACT GAT	753
TTG GTG CAG TTT CTG CTC TTC AAG TAT CAA ATG AAG GAG CCG	795
ATC ACA AAG GCA GAA ATA CTG GAG AGT GTC ATA AAA AAT TAT	837
GAA GAC CAC TTC CCT TTG TTG TTT AGT GAA GCC TCC GAG TGC	879
ATG CTG CTG GTC TTT GGC ATT GAT GTA AAG GAA GTG GAT CC	920

(2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1107 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-11 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGAGAACAGG CCAACCTGGA GGACAGGAGT CCCAGGAGAA CCCAGAGGAT	50
CACTGGAGGA GAACAAGTGT AAGTAGGCCT TTGTTAGATT CTCCATGGTT	100
CATATCTCAT CTGAGTCTGT TCTCACGCTC CCTCTCTCCC CAGGCTGTGG	150
GGCCCCATCA CCCAGATATT TCCCACAGTT CGGCCTGCTG ACCTAACCAG	200
AGTCATCATG CCTCTTGAGC AAAGAAGTCA GCACTGCAAG CCTGAGGAAG	250
CCTTCAGGCC CAAGAAGAAG ACCTGGGCCT GGTGGGTGCA CAGGCTCTCC	300
AAGCTGAGGA GCAGGAGGCT GCCTTCTTCT CCTCTACTCT GAATGTGGGC	350
ACTCTAGAGG AGTTGCCTGC TGCTGAGTCA CCAAGTCCTC CCCAGAGTCC	400
TCAGGAAGAG TCCTTCTCTC CCACTGCCAT GGATGCCATC TTTGGGAGCC	450
TATCTGATGA GGGCTCTGGC AGCCAAGAAA AGGAGGGGCC AAGTACCTCG	500
CCTGACCTGA TAGACCCTGA GTCCTTTTCC CAAGATATAC TACATGACAA	550
GATAATTGAT TTGGTTTCATT TATTCTCCGC AAGTATCGAG TCAAGGGGCT	600
GATCACAAG GCAGAA	616
ATG CTG GGG AGT GTC ATC AAA AAT TAT GAG GAC TAC TTT CCT	658
GAG ATA TTT AGG GAA GCC TCT GTA TGC ATG CAA CTG CTC TTT	700
GGC ATT GAT GTG AAG GAA GTG GAC CCC ACT AGC CAC TCC TAT	742
GTC CTT GTC ACC TCC CTC AAC CTC TCT TAT GAT GGC ATA CAG	784
TGT AAT GAG CAG AGC ATG CCC AAG TCT GGC CTC CTG ATA ATA	826
GTC CTG GGT GTA ATC TTC ATG GAG GGG AAC TGC ATC CCT GAA	868
GAG GTT ATG TGG GAA GTC CTG AGC ATT ATG GGG GTG TAT GCT	910
GGA AGG GAG CAC TTC CTC TTT GGG GAG CCC AAG AGG CTC CTT	952
ACC CAA AAT TGG GTG CAG GAA AAG TAC CTG GTG TAC CGG CAG	994
GTG CCC GGC ACT GAT CCT GCA TGC TAT GAG TTC CTG TGG GGT	1036
CCA AGG GCC CAC GCT GAG ACC AGC AAG ATG AAA GTT CTT GAG	1078
TAC ATA GCC AAT GCC AAT GGG AGG GAT CC	1107

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(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2150 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: smage-I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

08919559-104797

TCTGTCTGCA	TATGCCTCCA	CTTGTGTGTA	GCAGTCTCAA	ATGGATCTCT	50
CTCTACAGAC	CTCTGTCTGT	GTCTGGCACC	CTAAGTGGCT	TTGCATGGGC	100
ACAGGTTTCT	GCCCCTGCAT	GGAGCTTAAA	TAGATCTTTC	TCCACAGGCC	150
TATACCCCTG	CATTGTAAGT	TTAAGTGGCT	TTATGTGGAT	ACAGGTCTCT	200
GCCCTTGAT	GCAGGCCTAA	GTTTTTCTGT	CTGCTTAACC	CCTCCAAGTG	250
AAGCTAGTGA	AAGATCTAAC	CCACTTTTGG	AAGTCTGAAA	CTAGACTTTT	300
ATGCAGTGGC	CTAACAAAGT	TTAATTTCTT	CCACAGGGTT	TGCAGAAAAG	350
AGCTTGATCC	ACGAGTTCAG	AAGTCCTGGT	ATGTTCTTAG	AAAG	394
ATG TTC TCC TGG AAA GCT TCA AAA GCC AGG TCT CCA TTA AGT	436				
CCA AGG TAT TCT CTA CCT GGT AGT ACA GAG GTA CTT ACA GGT	478				
TGT CAT TCT TAT CCT TCC AGA TTC CTG TCT GCC AGC TCT TTT	520				
ACT TCA GCC CTG AGC ACA GTC AAC ATG CCT AGG GGT CAA AAG	562				
AGT AAG ACC CGC TCC CGT GCA AAA CGA CAG CAG TCA CGC AGG	604				
GAG GTT CCA GTA GTT CAG CCC ACT GCA GAG GAA GCA GGG TCT	646				
TCT CCT GTT GAC CAG AGT GCT GGG TCC AGC TTC CCT GGT GGT	688				
TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA	730				
GGT GTA TCC TGC ACA GGC TCT GGT ATA GGT GGT AGA AAT GCT	772				
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA	814				
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG	856				
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG	898				
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT	940				
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA	982				
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG	1024				
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA	1066				
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG	1108				
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC	1150				
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA	1192				
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG	1234				
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG	1276				
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC	1318				
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA	1360				
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT	1402				
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT	1444				
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA	1486				
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT	1528				
AAC ATG TAG	1537				
TTGAGTCTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG	1587				
AGAGTTCATA GCCTACCAGA ACCAACATGC ATCCATTCTT GGCCTGTTAT	1637				
ACATTAGTAG AATGGAGGCT ATTTTTGTGA CTTTTCAAAT GTTTGTTTAA	1687				
CTAAACAGTG CTTTTTGCCA TGCTTCTTGT TAACTGCATA AAGAGGTAAC	1737				

TGTCACCTTGT	CAGATTAGGA	CTTGTTTTGT	TATTTGCAAC	AAACTGGAAA	1787
ACATTATTTT	GTTTTTACTA	AAACATTGTG	TAACATTGCA	TTGGAGAAGG	1837
GATTGTCATG	GCAATGTGAT	ATCATACAGT	GGTGAAACAA	CAGTGAAGTG	1887
GGAAAGTTTA	TATTGTTAAT	TTTGAAAATT	TTATGAGTGT	GATTGCTGTA	1937
TACTTTTTTC	TTTTTTGTAT	AATGCTAAGT	GAAATAAAGT	TGGATTTGAT	1987
GACTTTACTC	AAATTCATTA	GAAAGTAAAT	CGTAAAACTC	TATTACTTTA	2037
TTATTTTCTT	CAATTATGAA	TTAAGCATTG	GTTATCTGGA	AGTTTCTCCA	2087
GTAGCACAGG	ATCTAGTATG	AAATGTATCT	AGTATAGGCA	CTGACAGTGA	2137
GTTATCAGAG	TCT				2150

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2099 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: smage-II

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACCTTATTGG	GTCTGTCTGC	ATATGCCTCC	ACTTGTGTGT	AGCAGTCTCA	50
AATGGATCTC	TCTCTACAGA	CCTCTGTCTG	TGTCTGGCAC	CCTAAGTGGC	100
TTTGCATGGG	CACAGGTTTC	TGCCCCTGCA	TGGAGCTTAA	ATAGATCTTT	150
CTCCACAGGC	CTATACCCCT	GCATTGTAAG	TTTAAGTGGC	TTTATGTGGA	200
TACAGGTCTC	TGCCCTTGTA	TGCAGGCCTA	AGTTTTTCTG	TCTGCTTAGC	250
CCCTCCAAGT	GAAGCTAGTG	AAAGATCTAA	CCCACTTTTG	GAAGTCTGAA	300
ACTAGACTTT	TATGCAGTGG	CCTAACAAGT	TTTAATTTCT	TCCACAGGGT	350
TTGCAGAAAA	GAGCTTGATC	CACGAGTTTC	GAAGTCCTGG	TATGTTCTTA	400
GAAAGATGTT	CTCCTGGAAA	GCTTCAAAAG	CCAGGTCTCC	ATTAAGTCCA	450
AGGTATTCTC	TACCTGGTAG	TACAGAGGTA	CTTACAGGTT	GTCATTCTTA	500
TCTTTCCAGA	TTCTGTCTG	CCAGCTCTTT	TACTTCAGCC	CTGAGCACAG	550
TCAACATGCC	TAGGGGTCAA	AAGAGTAAGA	CCCGCTCCCG	TGCAAAACGA	600
CAGCAGTCAC	GCAGGGAGGT	TCCAGTAGTT	CAGCCCACTG	CAGAGGAAGC	650
AGGGTCTTCT	CCTGTTGACC	AGAGTGCTGG	GTCCAGCTTC	CCTGGTGGTT	700
CTGCTCCTCA	GGGTGTGAAA	ACCCCTGGAT	CTTTTGGTGC	AGGTGTATCC	750
TGCACAGGCT	CTGGTATAGG	TGGTAGAAAT	GCTGCTGTCC	TGCCTGATAC	800
AAAAAGTTCA	GATGGCACCC	AGGCAGGGAC	TTCCATTTCAG	CACACACTGA	850
AAGATCCTAT	CATGAGGAAG	GCTAGTGTGC	TGATAGAATT	CCTGCTAGAT	900
AAGTTTAAGA	TGAAAGAAGC	AGTTACAAGG	AGTGAAATGC	TGGCAGTAGT	950
TAACAAGAAG	TATAAGGAGC	AATTCCCTGA	GATCCTCAGG	AGAACTTCTG	1000
CACGCCTAGA	ATTAGTCTTT	GGTCTTGAGT	TGAAGGAAAT	TGATCCCAGC	1050
ACTCATTCCCT	ATTTGCTGGT	AGGCAAACCTG	GGTCTTTCCA	CTGAGGGAAG	1100
TTTGAGTAGT	AACTGGGGGT	TGCCTAGGAC	AGGTCTCCTA	ATGTCTGTCC	1150
TAGGTGTGAT	CTTCATGAAG	GGTAACCGTG	CCACTGAGCA	AGAGGTCTGG	1200
CAATTTCTGC	ATGGAGTGGG	GGTATATGCT	GGGAAGAAGC	ACTTGATCTT	1250
TGGCGAGCCT	GAGGAGTTTA	TAAGAGATGT	AGTGCGGGAA	AATTACCTGG	1300
AGTACCGCCA	GGTACCTGGC	AGTGATCCCC	CAAGCTATGA	GTTCCCTGTGG	1350
GGACCCAGAG	CCCATGCTGA	AACAACCAAG	ATGAAAGTCC	TGGAAGTTTT	1400
AGCTAAAGTC	AATGGCACAG	TCCCTAGTGC	CTTCCCTAAT	CTCTACCACT	1450
TGGCTCTTAG	AGATCAGGCA	GGAGGGGTGC	CAAGAAGGAG	AGTTCAAGGC	1500

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AAGGGTGTTC	ATTCCAAGGC	CCCATCCCAA	AAGTCCTCTA	ACATGTAGTT	1550
GAGTCTGTTC	TGTTGTGTTT	GAAAAACAGT	CAGGCTCCTA	ATCAGTAGAG	1600
AGTTCATAGC	CTACCAGAAC	CAACATGCAT	CCATTCTTGG	CCTGTTATAC	1650
ATTAGTAGAA	TGGAGGCTAT	TTTTGTTACT	TTTCAAATGT	TTGTTTAACT	1700
AAACAGTGCT	TTTGCCATG	CTTCTTGTTA	ACTGCATAAA	GAGGTAAGTG	1750
TCACTTGTC	GATTAGGACT	TGTTTTGTTA	TTTGCAACAA	ACTGGAAAAC	1800
ATTATTTTGT	TTTTACTAAA	ACATTGTGTA	ACATTGCATT	GGAGAAGGGA	1850
TTGTCATGGC	AATGTGATAT	CATACAGTGG	TGAAACAACA	GTGAAGTGGG	1900
AAAGTTTATA	TTGTTAGTTT	TGAAAATTTT	ATGAGTGTGA	TTGCTGTATA	1950
CTTTTTTCTT	TTTGTATATA	TGCTAAGTGA	AATAAAGTTG	GATTTGATGA	2000
CTTTACTCAA	ATTCATTAGA	AAGTAAATCA	TAAAACTCTA	TTACTTTATT	2050
ATTTTCTTCA	ATTATTAATT	AAGCATTGGT	TATCTGGAAG	TTTCTCCAG	2099

(2) INFORMATION FOR SEQUENCE ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acids

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Ala Asp Pro Thr Gly His Ser Tyr

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03/17/97

TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR
REJECTION ANTIGENS AND USES THEREOF

This application is a continuation-in-part of Serial Number 807,043, filed December 12, 1991, which is a continuation-in-part of Serial Number 764,364, filed September 23, 1991, which is a continuation-in-part of Serial Number 728,838, filed July 9, 1991, which is a continuation-in-part of Serial Number 705,702, filed May 23, 1991, and now abandoned.

10 FIELD OF THE INVENTION

This invention relates in general to the field of immunogenetics as applied to the study of oncology. More specifically, it relates to the study and analysis of mechanisms by which tumors are recognized by the organism's immune system such as through the presentation of so-called tumor rejection antigens, and the expression of what will be referred to herein as "tumor rejection antigen precursors".

BACKGROUND AND PRIOR ART

20 The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

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Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See 10 Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar 20 results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

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The family of tum⁻ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum⁻ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum⁺" cells). When these tum⁺ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum⁻"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum⁻ variants fail to form progressive tumors because they elicit an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum⁻" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum⁻ cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory

which permits them to resist subsequent challenge to the same tum^r variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response
10 against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized
20 by cytotoxic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and

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the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This

10 type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and a class of antigens, referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are

20 incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum⁻ variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum⁻ antigens are

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only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum^+ , such as the line referred to as "P1", and can be provoked to produce tum^- variants. Since the tum^- phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum^- cell lines as compared to their tum^+ parental lines, and this difference can be exploited to locate the gene of interest in tum^- cells. As a result, it was found that genes of tum^- variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the tum^- antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d , P35 by D^d and P198 by K^d .

It has now been found that the genes which code for the molecules which are processed to form the presentation tumor rejection antigens (referred to as "tumor rejection antigen precursors", "precursor molecules" or "TRAPs" hereafter), are not expressed in most normal adult tissues but are expressed in tumor cells. Genes which code for the TRAPs have now been isolated and cloned, and represent a portion of the invention disclosed herein.

The gene is useful as a source for the isolated and purified tumor rejection antigen precursor and the TRA themselves, either of which can be used as an agent for treating the cancer for which the antigen is a "marker", as well as in various diagnostic and surveillance approaches to oncology, discussed infra. It is known, for example, that tum⁻ cells can be used to generate CTLs which lyse cells presenting different tum⁻ antigens as well as tum⁺ cells. See, e.g., Maryanski et al., Eur. J. Immunol 12: 401 (1982); and Van den Eynde et al., Modern Trends in Leukemia IX (June 1990), the disclosures of which are incorporated by reference. The tumor rejection antigen precursor may be expressed in cells transfected by the gene, and then used to generate an immune response against a tumor of interest.

In the parallel case of human neoplasms, it has been observed that autologous mixed lymphocyte-tumor cell cultures ("MLTC" hereafter) frequently generate responder lymphocytes which lyse autologous tumor cells and do not lyse natural killer targets, autologous EBV-transformed B cells, or autologous fibroblasts (see Anichini et al., Immunol. Today 8: 385-389 (1987)). This response has been particularly well studied for melanomas, and MLTC have been carried out either with peripheral blood cells or with tumor infiltrating lymphocytes. Examples of the literature in this area including Knuth et al., Proc. Natl. Acad. Sci. USA 86: 2804-2802 (1984); Mukherji et al., J. Exp. Med.

158: 240 (1983); Hérin et al., Int. J. Canc. 39: 390-396 (1987); Topalian et al., J. Clin. Oncol. 6: 839-853 (1988). Stable cytotoxic T cell clones ("CTLs" hereafter) have been derived from MLTC responder cells, and these clones are specific for the tumor cells. See Mukherji et al., supra, Hérin et al., supra, Knuth et al., supra. The antigens recognized on tumor cells by these autologous CTLs do not appear to represent a cultural artifact, since they are found on fresh tumor cells. Topalian et al., supra; Degiovanni et al., Eur. J. Immunol. 20: 1865-1868 (1990). These observations, coupled with the techniques used herein to isolate the genes for specific murine tumor rejection antigen precursors, have led to the isolation of nucleic acid sequences coding for tumor rejection antigen precursors of TRAs presented on human tumors. It is now possible to isolate the nucleic acid sequences which code for tumor rejection antigen precursors, including, but not being limited to those most characteristic of a particular tumor, with ramifications that are described infra. These isolated nucleic acid sequences for human tumor rejection antigen precursors and applications thereof, as described infra, are also the subject of this invention.

These and various other aspects of the invention are elaborated upon in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts detection of transfectants expressing antigen P815A.

Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

Figure 6 shows the results obtained when cells were transfected with the gene from P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment sequence which also express the antigen.

Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

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Figure 11 presents the data of Figure 13 in table form.

Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 is cDNA for part of gene P1A.

SEQ ID NO: 2 presents coding region of cDNA for gene P1A.

10 SEQ ID NO: 3 shows non coding DNA for P1A cDNA which is 3' to the coding region of SEQ ID NO: 2.

SEQ ID NO: 4 is the entire sequence of cDNA for P1A.

SEQ ID NO: 5 is the genomic DNA sequence for P1A.

SEQ ID NO: 6 shows the amino acid sequence for the antigenic peptides for P1A TRA. The sequence is for cells which are A⁺ B⁺, i.e., express both the A and B antigens.

SEQ ID NO: 7 is a nucleic acid sequence coding for antigen E.

SEQ ID NO: 8 is a nucleic acid sequence coding for MAGE-1.

20 SEQ ID NO: 9 is the gene for MAGE-2.

SEQ ID NO: 10 is the gene for MAGE-21.

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SEQ ID NO: 11 is cDNA for MAGE-3.

SEQ ID NO: 12 is the gene for MAGE-31.

SEQ ID NO: 13 is the gene for MAGE-4.

SEQ ID NO: 14 is the gene for MAGE-41.

SEQ ID NO: 15 is cDNA for MAGE-4.

SEQ ID NO: 16 is cDNA for MAGE-5.

SEQ ID NO: 17 is genomic DNA for MAGE-51.

SEQ ID NO: 18 is cDNA for MAGE-6.

SEQ ID NO: 19 is genomic DNA for MAGE-7.

10 SEQ ID NO: 20 is genomic DNA for MAGE-8.

SEQ ID NO: 21 is genomic DNA for MAGE-9.

SEQ ID NO: 22 is genomic DNA for MAGE-10.

SEQ ID NO: 23 is genomic DNA for MAGE-11.

SEQ ID NO: 24 is genomic DNA for smage-I.

SEQ ID NO: 25 is genomic DNA for smage-II.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following

examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection, 10^6 cells of P1.HTR were mixed with $2-4 \times 10^6$ cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosure of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

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present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

10

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum^r antigens.

20

Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 μ g of cellular DNA and 3 μ g of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 μ l of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 μ l 1M CaCl_2 .

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4 , adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells (5×10^6) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm² tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing 8×10^6 cells in 40 ml of medium. In order to estimate the number of transfectants, 1×10^6 cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about 6×10^4 cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells (2×10^3 - 4×10^3 per well), and chromium release

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was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

10 In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single
20 microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

10 Prior work had shown that genes coding for tum⁻ antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately 9×10^5

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ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of 2×10^8 cells/ml ($OD_{600}=0.8$), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of 5×10^6 PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of H ₂ B ^T transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

10 Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

20 All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA⁺ mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A⁺ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A⁺ RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5' end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

10 The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

Example 8

20 The Northern analysis described supra suggested that the 5' end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3' primer corresponding to positions 286-266 and a 5' primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

10 The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by
20 Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

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for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

10 With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions - 0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

20 Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A⁻B⁺", rather than the normal "P1A". The only difference
10 between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

20 The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

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J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2^d haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

10 Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

Example 12

20 The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No. of clones lysed by the CTL/ no. of H-2B ⁺ clones*	
	CTL anti-A	CTL anti-B
DAP (H-2 ^k)	0/208	0/194
DAP + K ^d	0/165	0/162
DAP + D ^d	0/157	0/129
DAP + I ^d	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2^d class I genes as indicated.

*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A⁺ B⁺ (i.e., characteristic of cells which express both the A and B antigens), and those which are A⁻ B⁺ were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

10 The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

20 In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E⁻. This subclone is also HPRT⁻, (i.e., sensitive to HAT medium: 10⁻⁴ M hypoxanthine, 3.8 x 10⁻⁷ aminopterin, 1.6 x 10⁻⁵ M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

10 The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneo β , as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

20 Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 μ g) and plasmid DNA (6 μ g) were mixed in 940 μ l of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 μ l of 1M CaCl₂ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na₂HPO₄, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

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temperature, after which they were applied to 80 cm² tissue culture flasks which had been seeded 24 hours previously with 3x10⁶ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at 4x10⁶ cells per 80 cm² flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

10

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

20

After 10 days, wells contained approximately 6x10⁴ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 µl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 µl) was harvested and examined

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for TNF concentration, for reasons set forth in the following example.

Example 17

10 The size of the mammalian genome is 6×10^6 kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interest could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

20 As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 (4×10^4) had readhered, the CTLs and IL-2 were added thereto. The 50 μ l of supernatant was removed 24 hours later and transferred to a microplate containing 3×10^4 W13 (WEHI-164 clone 13;

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Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μ l of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μ g of actinomycin D at 37% in an 8% CO₂ atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF- β in RPMI 1640 were added to target cell controls.

10 The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μ l of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were
20 incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[1 - \frac{100 - (\text{OD}_{570} \text{ sample well})}{\text{OD}_{570} \text{ well} + \text{medium}} \right]$$

To carry out the selection, 10^6 cells of P1.HTR were mixed with $2-4 \times 10^6$ cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosure of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

10 Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum⁻ antigens.

20 Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 µg of cellular DNA and 3 µg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 ul 1M CaCl₂.

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4 , adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells (5×10^6) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm² tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing 8×10^6 cells in 40 ml of medium. In order to estimate the number of transfectants, 1×10^6 cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about 6×10^4 cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells (2×10^3 - 4×10^3 per well), and chromium release

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was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

10 In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single
20 microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

Example 4

10 Prior work had shown that genes coding for tum-
antigens could be recovered directly from transfectants
obtained with a cosmid library. See DePlaen et al., Proc.
Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure
was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., *supra*. Approximately 9×10^5

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of 2×10^8 cells/ml ($OD_{600}=0.8$), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of 5×10^6 PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of HmB ^r transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

10 Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

20 All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

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Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA⁺ mRNA using oligodT cellulose column chromatography.

10

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

20

When this protocol was carried out using P1.HTR poly A⁺ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A⁺ RNA from the cell line. This yielded

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a clone with a 1kb insert, suggesting a missing 5' end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

10 The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

Example 8

20 The Northern analysis described supra suggested that the 5' end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3' primer corresponding to positions 286-266 and a 5' primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

10 The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by
20 Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions - 0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A⁻B⁺", rather than the normal "P1A". The only difference
10 between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

20 The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

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J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2^d haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

10 Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

Example 12

20 The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

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Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No. of clones lysed by the CTL/ no. of H-mB ⁺ clones*	
	CTL anti-A	CTL anti-B
DAP (H-2 ^k)	0/208	0/194
DAP + K ^d	0/165	0/162
DAP + D ^d	0/157	0/129
DAP + L ^d	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2^d class I genes as indicated.

*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A⁺ B⁺ (i.e., characteristic of cells which express both the A and B antigens), and those which are A⁻ B⁺ were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

10 The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

20 In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E⁻. This subclone is also HPRT⁻, (i.e., sensitive to HAT medium: 10⁻⁴ M hypoxanthine, 3.8 x 10⁻⁷ aminopterin, 1.6 x 10⁻⁵ M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

10 The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneo⁸, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

20 Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 µg) and plasmid DNA (6 µg) were mixed in 940 µl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 µl of 1M CaCl₂ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na₂HPO₄, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

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temperature, after which they were applied to 80 cm² tissue culture flasks which had been seeded 24 hours previously with 3x10⁶ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at 4x10⁶ cells per 80 cm² flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

10

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

20

After 10 days, wells contained approximately 6x10⁴ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 µl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 µl) was harvested and examined

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for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is 6×10^6 kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interest could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 (4×10^4) had readhered, the CTLs and IL-2 were added thereto. The 50 μ l of supernatant was removed 24 hours later and transferred to a microplate containing 3×10^4 W13 (WEHI-164 clone 13;

Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μ l of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μ g of actinomycin D at 37% in an 8% CO₂ atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF- β in RPMI 1640 were added to target cell controls.

10 The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μ l of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were
20 incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[1 - \frac{100 - (\text{OD}_{570} \text{ sample well})}{\text{OD}_{570} \text{ well} + \text{medium}} \right]$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E^+/E^- cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E^- cells (4×10^6 cells/group) were tested following transfection, and 7×10^4 independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard ^{51}Cr release assay, and were found to be lysed as efficiently as the original E^+ cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E^+ contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B^- and C^- , just like the recipient cell MEL2.2. It was also found to be $HPRT^-$, using standard selection procedures. All E^+ cells used in the work described herein, however, were $HPRT^+$.

10 It was also possible that an E^+ revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfection with pSVtkneo β , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo β sequences. Wölfel et al., supra, has shown this to be true. If a normally E^- cell is transfected with pSVtkneo β , then
20 sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo β sequences. If a normally E^+ cell transfected with pSVtkneo β is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin

resistance; however, Southern blot analysis showed loss of several neo^r sequences in the variants, showing close linkage between the E gene and neo^r gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

Example 20

The E^+ subclone MZ2-MEL 4B was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phase components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so we rescued the transfected sequence by ligating DNA of the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI

fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI/SmaI digested DNA). The band is absent from E⁻ antigen loss variants of MZ2-MEL, as seen in Figure 12.

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The sequence for the E antigen precursor gene has been determined, and is presented herein:

	1	10	20	30	40	50	60
3	GGATCCAGGC	CCTGCCAGGA	AAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGCTCATCC	60
61	ACTGCAATGAG	AGTGGGSAATG	TACACAGATC	CAGCCCAACC	TCCCTGATAGC	ACTGAGAAAGC	120
121	CAGGGCTGTG	CTTGGCGTCT	GCACCCCTGAG	GGCCCGTGA	TTCCCTCTTC	TGSACTCCA	180
181	GGAAACAGGC	AGTGAAGGCT	TGGTCTGAGA	CAGTATCCCTC	AGGTCAACAG	GCAGAGGATG	240
241	CACAGGCTGT	GGCAGCAATG	AAATGTTTGGC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
301	CAGGACACAT	AGGATCCAC	AGAGTCTGAC	CTCACCTCCC	TACTGTCAAT	CCTGTAGAA	360
361	CGACCTCTGC	TGGCCGGCTG	TACCTGTAGT	ACCCCTCTAC	TTCCCTCTTC	AGGTTTCTAG	420
421	GGGATAGGTC	AACCCAGAGC	ACAGGAATCC	CTGGAGGCCA	CAGAGGAGCA	CCAAAGAGAA	480
481	GAATCTGTAA	TAGGCTCTTG	TTAGAGTCTC	CAAGGTTTCTAG	TTCTCAGCTG	AGGCTCTCTA	540
541	CACACTCCCT	CTCTCCCCAG	GGCTGTGGGT	CTTCAATTGCC	CAGCTCCCTGC	CCACACTCCCT	600
601	GCCTGCTGCC	CTGAAGAGAG	TCAATCATGTC	TCTTGAGCAG	AGGAGTCTGC	ACTGCAAGGC	660
661	TGAGGAAAGC	CTTGAAGGCC	AAACAAGAGC	CTTGGGCTGG	TGTGTGTGCA	GGCTGCCACC	720
721	TCCCTCCCTCT	CTCCCTCTGGT	CCTGGGCAAC	CTGGAGGAGG	TGCCCACTGC	TGGGTCAACA	780
781	GAATCCCTCCC	AGAGTCTCTA	GGGAGCCTCC	GCCTTCCCA	CTACCATCAA	CTTCACTCGA	840
841	CAGAGGCAAC	CCAGTGAAGG	TTCCAGCAGC	CGTGAAGAGG	AGGGGCCAAG	CACCTCTTGT	900
901	ATCCTGAGAT	CCTTGTTCGG	AGCAATAATC	ACTAAGGAGG	TGGCTGAATT	GGTTGATTTT	960
961	CTGCTCCCTCA	AATATCGAGC	CAGGAGGCCA	GTCAAAAGG	CAGAAATGCT	GGAGAGTGTG	1020
1021	ATCAAAATTT	ACAAGCACTG	TTTTCTTGAG	ATCTTGGCCA	AAAGCTCTGA	GTCCCTTGCA	1080
1081	CTGGTCTTTG	GCATTGAGCT	GAAAGGAGCA	GACCCCAACG	GCCACTCCTA	TGTCTCTTGT	1140
1141	ACCTGCCCTAG	GTCTCTCCTA	TGAATGCCCTG	CTGGGTGATA	ATCAGATCAT	GGCCAAAGCA	1200
1201	GGCTTCTCTGA	TAAATTGTCT	GGTCATGATT	GCAATGGAGG	GGGCCCATGC	TCTTGAGGAG	1260
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCTAT	1320
1321	GGGAGGCCCA	GGAGGCTGCT	CACCCAAAGT	TTGGTGCAAG	AAAGTACCT	GGAGTACGGC	1380
1381	AGGTGCCGGA	CAGTGAATCC	GCACCTCTATG	AGTTCTCTGT	GGTCCAAAGG	GGCCTCGCTG	1440
1441	AAACCAAGCTA	TGTGAAGATC	CTTGAGTATG	TGAATCAAGG	CAGTGAAGCA	GTTCGCTTTT	1500
1501	TCTTCCCATC	CCTGCCGTGA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1560
1561	TTGCAGGCCA	GGCCAGTGGG	AGGGGSACTG	GGCCATGCA	CCTTCCAGGG	CGGCTCCAG	1620
1621	CAGCTTCCCC	TGCCCTCGTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGGTCAAT	1680
1681	GTCTCTAGTA	GTAGGTTTCT	GTTCATATGG	GTGACTTGGG	GATTTATCTT	TGTTCTCTTT	1740
1741	TGGAAATGTT	CAAAATGTTT	TTTTTAAGGG	ATGTTTGAAT	GAACTTCAGC	ATCCAAATTT	1800
1801	ATGAATGACA	GCAGTCAAC	AGTTCTGTGT	ATATAGTTTA	AGGTTAAGAG	TCTTGTGTTT	1860
1861	TATTCAGATT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAAATACAGC	AGTGGAAATA	1920
1921	GTACTTAGAA	ATGTGAJAAA	TGAGCAGTAA	AAATAGATGAG	ATAAAGAACT	AAAGAAATTA	1980
1981	AGAGATAGTC	AAATCTTTGGC	TTATACCTCA	GTCTATTTCTG	TAAATTTTCT	AAAGATATAT	2040
2041	GCATACCTGG	ATTTCTCTTG	CTTCTTTGAG	AAATGAAGAG	AAATTAATTC	TGAATTAAGA	2100
2101	ATCTCTCTCTG	TTCACTGGCT	CTTTCTCTCT	CCATCTGCTG	AGCATCTGCT	TTTTTGAAGG	2160
2161	CCCTGGGTAA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGTTCTG	2220
2221	AGATCTAGG	AGCTGCAATC	ACCTAATCCA	GGTGGCAAGA	TGTCTCTTAA	AGATGTAGGG	2280
2281	AAAGTGAAG	AGGGGCTGAG	GGTGTGGGTC	TCCGGGTGAG	AGTGTGAGAG	TGTCAATGCC	2340
2341	CTGACCTGGG	GCATTTTGGG	CTTTGGGAAA	CTGCAATTC	TTCTGGGGGA	GTGATTGTA	2400
2401	ATGATCTTGG	GTGATCC					2418
	1	10	20	30	40	50	60

Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E⁺" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5' end of the cDNA. The three exons comprise 65, 73, and 1551 base pairs. An ATG is located at position 66 of exon 3, followed by an 828 base pair reading frame.

Example 22

To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E⁻ cells. Figure 8 shows the boundaries of the three segments.

Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

10 The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, "mage -1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a

20 family of molecules, and the nucleic acids coding for them. These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments which follow indicate, however, the members of the MAGE family are not at all restricted to melanoma tumors;

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rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the mage-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2.

Amplification by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with the DNA of the E⁻ variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E⁺ melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene mage-1 by various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals (Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these culture cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2 proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes mage-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligo- nucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers

corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than 1/300th that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also expressed all three genes whereas others expressed only mage-2 and 3 or only mage-3. It is impossible to exclude formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but that of yet another closely related gene that would share the sequence of the priming and hybridizing oligonucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this point.

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Example 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneo β . Three of them yielded neo^r transfectants that

10 stimulated TNF release by anti-E CTL clone 82/30, which is CD8⁺ (Figure 10). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL also showed high sensitivity to lysis by these

20 anti-E CTL. These two melanomas were those that expressed mage-1 gene (Figure 13). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes

mage 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E⁻ cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation of antigen -E precursor DNA, the F⁻ variant was transfected with genomic DNA from F⁺ cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-

F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

Example 28

Following identification of F⁺ cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F⁺ cell line MZ2-MEL.43 was prepared, again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 genitacin resistant transfectants.

Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen M22-E, was labelled with 32p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone M22-MEL2.2. Hybridization conditions included 50 μ l/cm² of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with [α^{32} p]dCTP (2-3000

Ci/mole), at 3×10^6 cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

10 In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

Example 30

20 The cDNA coding for mage 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express mage 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for mage 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as mage 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which

showed homology to mage 1 but not mage 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "mage 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT), and CHO10: (GAAGAGGAGGGGCCAAG). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1 μ g of RNA was diluted to a total volume of 20 μ l, using 2 μ l of 10x PCR buffer, 2 μ l of each of 10 mM dNTP, 1.2 μ l of 25 mM $MgCl_2$, 1 μ l of an 80 mM solution of CHO9, described supra, 20 units of RNasin, and 200 units of M-MLV reverse transcriptase. This was followed by incubation for 40 minutes at 42°C. PCR amplification followed, using 8 μ l of 10x PCR buffer, 4.8 μ l of 25 mM $MgCl_2$, 1 μ l of CHO10, 2.5 units of *Thermus aquaticus* ("Taq") polymerase, and water to a total volume of 100 μ l. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten μ l of each reaction were then size fractionated on agarose gel,

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followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CH018 (TCTTGTATCCTGGAGTCC). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from mage 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to mage 4 and 5. These results indicate a sequence differing from previously identified mage 1, 2, 3, 4 and 5, and is named mage 6.

Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb mage 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for mage 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from mages 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded mage 8-11.

Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of mage 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether

synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr was shown to be best. The assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

Example 35

Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for

pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

The foregoing disclosure, including the examples, places many tools of extreme value in the hands of the skilled artisan. To begin, the examples identify and provide a methodology for isolating nucleic acid molecules which code for tumor rejection antigen precursors as well as the nucleic acid molecules complementary thereto. It is known that DNA exists in double stranded form, and that each of the two strands is complementary to the other. Nucleic acid hybridization technology has developed to the point where, given a strand of DNA, the skilled artisan can isolate its complement, or synthesize it.

"Nucleic acid molecule" as used herein refers to all species of DNA and RNA which possess the properties discussed supra. Genomic and complementary DNA, or "cDNA" both code for particular proteins, and as the examples directed to isolation of MAGE coding sequences show, this disclosure teaches the artisan how to secure both of these.

Similarly, RNA molecules, such as mRNA can be secured. Again, with reference to the skilled artisan, once one has a coding sequence in hand, mRNA can be isolated or synthesized.

Complementary sequences which do not code for TRAP, such as "antisense DNA" or mRNA are useful, e.g., in

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probing for the coding sequence as well as in methodologies for blocking its expression.

It will also be clear that the examples show the manufacture of biologically pure cultures of cell lines which have been transfected with nucleic acid sequences which code for or express the TRAP molecules. Such cultures can be used as a source for tumor rejection antigens, e.g., or as therapeutics. This aspect of the invention is discussed infra.

10 Cells transfected with the TRAP coding sequences may also be transfected with other coding sequences. Examples of other coding sequences include cytokine genes, such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene transfection is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of the cells in vivo. The art is well aware of therapies where interleukin transfectants have been administered to
20 subjects for treating cancerous conditions. In a particularly preferred embodiment, cells are transfected with sequences coding for each of (i) a TRAP molecule, (ii) an HLA/MHC molecule, and (iii) a cytokine.

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA,

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additional transfection may not be necessary although further transformation could be used to cause over-expression of the antigen. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that transfection with one additional sequence does not preclude further transfection with other sequences.

10 The term "biologically pure" as used in connection with the cell line described herein simply means that these are essentially free of other cells. Strictly speaking, a "cell line" by definition is "biologically pure", but the recitation will establish this fully.

20 Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for the TRAP of interest is operably linked to a promoter. The promoter may be a strong promoter, such as those well known to the art, or a differential promoter, i.e., one which is operative only in specific cell types. The expression vectors may also contain all or a part of a viral or bacterial genome, such as vaccinia virus or BCG. Such vectors are especially useful in preparing vaccines.

 The expression vectors may incorporate several coding sequences, as long as the TRAP sequence is contained therein. The cytokine and/or MHC/HLA genes discussed supra may be included in a single vector with the TRAP sequence. Where this is not desired, then an expression system may be

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provided, where two or more separate vectors are used where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-transfection is a well known technique, and the artisan in this field is expected to have this technology available for utilization. The vectors may be constructed so that they code for the TRA molecule directly, rather than the TRAP molecule. This eliminates the need for post-translational processing.

10 As the foregoing discussion makes clear, the sequences code for "tumor rejection antigen precursors" ("TRAPs") which, in turn, are processed into tumor rejection antigens ("TRAs"). Isolated forms of both of these categories are described herein, including specific examples of each. Perhaps their most noteworthy aspect is as vaccines for treating various cancerous conditions. The evidence points to presentation of TRAs on tumor cells, followed by the development of an immune response and deletion of the cells. The examples show that when various TRAs are
20 administered to cells, a CTL response is mounted and presenting cells are deleted. This is behavior characteristic of vaccines, and hence TRAPs, which are processed into TRAs, and the TRAs themselves may be used, either alone or in pharmaceutically appropriate compositions, as vaccines. Similarly, presenting cells may be used in the same manner, either alone or as combined with ingredients to yield pharmaceutical compositions. Additional materials which may be used as vaccines include

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isolated cells which present the TRA molecule on their surface, as well as TRAP fragments, mutated viruses, especially etiolated forms, and transfected bacteria. "Fragments" as used herein refers to peptides which are smaller than the TRA, but which possess the properties required of a vaccine, as discussed supra. Another vaccine comprises or consists of complexes of TRA and HLA molecule. Vaccines of the type described herein may be used preventively, i.e., via administration to a subject in an amount sufficient to prevent onset of a cancerous condition.

The generation of an immune response, be it T-cell or B-cell related, is characteristic of the effect of the presented tumor rejection antigen. With respect to the B-cell response, this involves, inter alia, the generation of antibodies to the TRA, i.e., which specifically bind thereto. In addition, the TRAP molecules are of sufficient size to render them immunogenic, and antibodies which specifically bind thereto are a part of this invention. These antibodies may be polyclonal or monoclonal, the latter being prepared by any of the well recognized methodologies for their preparation which need not be repeated here. For example, mAbs may be prepared using an animal model, e.g., a Balb/C mouse or in a test tube, using, e.g., EBV transformants. In addition, antiserum may be isolated from a subject afflicted with a cancerous condition where certain cells present a TRA. Such

antibodies may also be generated to epitopes defined by the interaction of TRA and HLA/MHC molecules.

Review of the foregoing disclosure will show that there are a number of facets to the system which may be referred to as "tumor rejection antigen presentation and recognition". Recognition of these phenomena has diagnostic consequences. For example, the existence of specific CTL clones, or antibodies to the TRA makes it possible to diagnose or monitor cancerous conditions (explained infra), by monitoring the CTLs in a sample from a subject, binding of antibodies to TRAs, or the activity of anti-TRA CTLs in connection with subject samples. Similarly, the expression of nucleic acid molecules for TRAPs can be monitored via amplification (e.g., "polymerase chain reaction"), anti-sense hybridization, probe technologies, and so forth. Various subject samples, including body fluids (blood, serum, and other exudates, e.g.), tissues and tumors may be so assayed.

A particular manner of diagnosis is to use an adaptation of the standard "tuberculin test" currently used for diagnosis of tuberculosis. This standard skin test administers a stable form of "purified protein derivative" or "PPD" as a diagnostic aid. In a parallel fashion, TRAs in accordance with this invention may be used in such a skin test as a diagnostic aid or monitoring method.

The term "cancerous condition" is used herein to embrace all physiological events that commence with the initiation of the cancer and result in final clinical

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manifestation. Tumors do not spring up "ab initio" as visible tumors; rather there are various events associated with the transformation of a normal cell to malignancy, followed by development of a growth of biomass, such as a tumor, metastasis, etc. In addition, remission may be conceived of as part of "a cancerous condition" as tumors seldom spontaneously disappear. The diagnostic aspects of this invention include all events involved in carcinogenesis, from the first transformation to malignancy of a single cell, through tumor development and metastasis, as well as remission. All are embraced herein.

Where "subject" is used, the term embraces any species which can be afflicted with a cancerous condition. This includes humans and non-humans, such as domesticated animals, breeding stock, and so forth.

There are therapeutic aspects of this invention as well. The efficacy of administration of effective amounts of TRAPs and TRAs as vaccines has already been discussed supra. Similarly, one may develop the specific CTLs in vitro and then administer these to the subject. Antibodies may be administered, either polyclonal or monoclonal, which specifically bind to cells presenting the TRA of interest. These antibodies may be coupled to specific antitumor agents, including, but not being limited to, methotrexate radio-iodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Thus, "targeted" antibody therapy is included herein, as is the

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application of deletion of the cancerous cells by the use of CTLs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

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Claims:

1. Isolated nucleic acid molecule which codes for a tumor rejection antigen precursor or is complementary to a nucleic acid molecule which codes for a tumor rejection antigen precursor.
2. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a tumor rejection antigen precursor.
3. Isolated nucleic acid molecule of claim 1, wherein said molecule codes for a human tumor rejection antigen precursor.
4. The isolated nucleic acid molecule of claim 1, wherein said molecule is complementary to a nucleic acid molecule which codes for tumor rejection antigen precursor.
5. The isolated nucleic acid molecule of claim 1, wherein said molecule is DNA.
6. The isolated nucleic acid molecule of claim 1, wherein said molecule is RNA.
7. The isolated nucleic acid molecule of claim 1, wherein said molecule is a gene.

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8. The isolated nucleic acid molecule of claim 5, wherein said DNA is genomic DNA.
9. The isolated nucleic acid molecule of claim 5, wherein said DNA is cDNA.
10. The isolated nucleic acid molecule of claim 6, wherein said RNA is mRNA.
11. The isolated nucleic acid molecule of claim 4, wherein said molecule hybridizes to isolated nucleic acid which codes for tumor rejection antigen precursor under stringent conditions.
12. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a MAGE antigen precursor or is complementary to a molecule which codes for a MAGE antigen precursor.
13. The isolated nucleic acid molecule of claim 12, wherein said MAGE antigen precursor is selected from the group consisting of mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.
14. The isolated nucleic acid molecule of claim 12, wherein said molecule codes for a MAGE antigen precursor.

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15. The isolated nucleic acid molecule of claim 12, wherein said molecule is complementary to a molecule which codes for a MAGE antigen precursor.
16. The isolated nucleic acid molecule of claim 12, wherein said molecule is DNA.
17. The isolated nucleic acid molecule of claim 12, wherein said molecule is RNA.
18. The isolated nucleic acid molecule of claim 12, wherein said molecule is a gene.
19. The isolated nucleic acid molecule of claim 16, wherein said DNA is genomic DNA.
20. The isolated nucleic acid molecule of claim 16, wherein said DNA is cDNA.
21. The isolated nucleic acid molecule of claim 17, wherein said RNA is mRNA.
22. The isolated nucleic acid molecule of claim 12, comprising a nucleotide sequence set forth in figure 9.

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23. The isolated nucleic acid molecule of claim 15, wherein said molecule hybridizes to a molecule which codes for a MAGE antigen precursor under stringent conditions.
24. Isolated nucleic acid molecule of claim 1, coding for a tumor rejection antigen precursor for mastocytoma.
25. Isolated nucleic acid molecule of claim 1, coding for tumor rejection antigen precursor P1A.
26. Isolated nucleic acid molecule of claim 1, having the nucleotide sequence of figure 5.
27. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 2.
28. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 12.
29. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 22.
30. Biologically pure culture of a cell line of claim 27, selected from the group consisting of P1A.T2 and P1A.TC3.1.

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31. Biologically pure culture of a highly transfectable cell line derived from a parent cell line which expresses at least one P815 tumor antigen, wherein said highly transfectable cell line does not express any of P815 tumor antigens A, B and C.
32. Biologically pure cell line of claim 31, comprising cell line PO.HTR.
33. Biologically pure culture of a cell line of claim 27, wherein said tumor rejection antigen precursor is a human tumor antigen precursor.
34. Biologically pure culture of a cell line of claim 33, wherein said human tumor antigen precursor is found in melanoma cells.

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35. Biologically pure cell line of claim 34, said tumor rejection antigen precursor is mage-1 and said isolated DNA has nucleic acid sequence:

	1	10	20	30	40	50	60	
1	GGATCCAGGC	CTGCCCAGCA	AAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGGTCATCC	60	
61	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCAACC	TCCTGCTAGC	ACTGAGAAAGC	120	
121	CAGGGGCTGTG	CTTGGGGTCT	GCACCCCTGAG	GGCCCGTGGG	TTCCCTCTTC	TGGAGCTCCA	180	
181	GGAAACAGGC	AGTGAGGCGT	TGGTCTGAGA	CAGTATCCCT	AGGTCAACAG	GCAGAGGATG	240	
241	CACAGGGTGT	GGCAGCAGTG	AAATGTTTGGC	CTGAATGCAC	ACCAAGGGGC	CCACCTGCCA	300	
301	CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	CCTGTAGAA	360	
361	CGACCTCTGC	TGGCCGGGCTG	TAACCTGAGT	ACCCCTCTAC	TTCCCTCCTC	AGGTCTTCAG	420	
421	GGGACAGGCG	AACCCAGAGG	ACAGGAATTC	CTGGAGGCGA	CAGAGGAGCA	CCAGGAGAA	480	
481	GATCTGTAA	TAGGCTCTTG	TTAGAGTCTC	CAAGGTTCAG	TTCTCAGCTG	AGGCTCTCTA	540	
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGGT	CTTCAATTGC	CAGCTCCTGC	CCACACTCCCT	600	
601	GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAAGCA	AGGAGTCTGC	ACTGCCAAGCC	660	
661	TGAGGAAGCC	CTTGAGGGCC	AACAAGAGGC	CTTGGGCTGG	TGTGTGTGCA	GGCTGCCACC	720	
721	TCCTCTCTCT	CTCTCTCTCT	CTTGGGCAAC	CTGGAGGAGG	TGCCCACTGC	TGGGTCAACA	780	
781	GATCTCTCCC	AGAGTCTCTA	GGGAGCCCTCC	GCCTTCCCA	CTACCATCAA	CTTCACTCGA	840	
841	CAGAGGCAAC	CCAGTGAAGG	TTCCAGCAGC	CGTGAAGAGG	AGGGGCCAAG	CACCTCTTGT	900	
901	ATCCTGGAGT	CTTGTCTCCG	AGCAATAATC	ACTAAGGAGG	TGCTGTATTT	GTTTGTCTTT	960	
961	CTGCTCTCTA	AAATATCGAG	CAGGAGGCCA	GTCAACAAAG	CAGAAATGCT	GGAGAGTGTG	1020	
1021	ATCAAAAAAT	ACAAGCACTG	TTTTCTTGAG	ATCTTCGSCA	AAAGCTCTGA	GTCTCTGCAG	1080	
1081	CTGGTCTCTG	GCAATTGAGT	GAAAGAAAGCA	GACCCCAACC	GCACTCTCTA	TGCTCTTGTG	1140	
1141	ACCTGCCCTAG	GTCTCTCTCT	TGAATGCTCT	CTGGGTGATA	ATCAGATCAT	GCCCAAGACA	1200	
1201	GGCTTCTCTA	TAATTTGCTT	GCTCATGATT	GCAATGGAGG	GGGGCCATGC	TCCTGAAGAG	1260	
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCTAT	1320	
1321	GGGGAGGCCA	GGAACTCTCT	CACCCAGAT	TTGGTGCAGG	AAAGTACCT	GGAGTACGGC	1380	
1381	AGGTGCCGGA	CAGTGAATCC	GCACCTATG	AGTTCTCTGT	GGGTCCAAAG	GCCCTCGCTG	1440	
1441	AAACCAGCTA	TGTGAAGATC	CTTGAGTATG	TGATCAAGGT	CAGTGCAGGA	GTTTCCGTTT	1500	
1501	TCTTCCCATC	CTTGCCTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAAGGAGTC	TGAGCATGAG	1560	
1561	TTGCAGGCCA	GGCCAGTGGG	AGGGGCACTG	GGCCAGTGCA	CTTCCAGGGG	CCGCGTCCAG	1620	
1621	CAGCTTCCCC	TGCCCTCGTG	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGGTCACT	1680	
1681	GTTCTCACTA	GTAGGTCTCT	GTTCTATTGG	GTGACTTGGG	GATTTATCTT	TGTTCTCTTT	1740	
1741	TGGAAATGTT	CAAAATGTTT	TTTTTAAGGG	ATGTTTGAAT	GAACTTCAGC	ATCCAAATTT	1800	
1801	ATGAATGACA	GCAATCACAC	AGTTCTGTGT	ATATAATTIA	AGGTAAGAG	TCTTGTGTTT	1860	
1861	TAATTCAGAT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAATAACAGC	AGTGAATCAA	1920	
1921	GTACTTAGAA	ATGTGAAAAA	TGAGCAGTAA	AAATAGATGAG	ATAAAGAACT	AAAGAAATTA	1980	
1981	AGAGATAGTC	AAATCTTTGG	TTATACCTCA	GTCTATTCTG	TAATAATTTT	AAAGTATAT	2040	
2041	GCATACCTGG	ATTTCTTTGG	CTTCTTTGAG	AAATGAAGAG	AAATTAATTC	TGAATTAAGA	2100	
2101	ATCTCTCTCT	TTCACTGGCT	CTTTCTCTCT	CCATGCACTG	AGCATCTGCT	TTTTGGAAAG	2160	
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGTTCTG	2220	
2221	AGAGTCTAGG	AGCTGCACTC	ACGTAAATCGA	GGTGGCAAGA	TGTCCTCTAA	AGATGTAGGG	2280	
2281	AAAGTGAGGA	GAGGGGTGAG	GGTGTGGGGC	TCCGGGTGAG	AGTGTGGAG	TGTCAATGCC	2340	
2341	CTGAGCTGGG	GCAATTTTGG	CTTTGGGAAA	CTGCAATTC	TCTGGGGGGA	GCTGATTGTA	2400	
2401	ATGATCTTGG	GTGCAATCC					2418	

36. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence coding for a cytokine.
37. The biologically pure culture of claim 36, wherein said cell line is further transfected by a nucleic acid sequence coding for an HLA molecule.
38. The biologically pure culture of claim 36, wherein said cytokine is an interleukin.
39. The biologically pure culture of claim 38, wherein said interleukin is IL-2.
40. The biologically pure culture of claim 38, wherein said interleukin is IL-4.
41. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence which codes for an MHC molecule or an HLA molecule.
42. The biologically pure culture of claim 27, wherein said cell line expresses an MHC or HLA molecule which presents a tumor rejection antigen derived from a tumor rejection antigen precursor (TRAP), wherein said TRAP is coded for by a nucleic acid sequence transfected into said cell line.

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43. The biologically pure culture of claim 27, wherein said culture is non-proliferative.
44. The biologically pure culture of claim 27, wherein said cell line is a fibroblast cell line.
45. Transfected bacteria containing the nucleic acid sequence of claim 2.
46. Mutated virus containing the nucleic acid sequence of claim 2.
47. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 2 operably linked to a promoter.
48. Expression vector useful in transfecting a cell comprising a nucleic acid sequence coding for a tumor rejection antigen operably linked to a promoter.
49. Expression vector of claim 47, wherein said promoter is a strong promoter.
50. Expression vector of claim 47, wherein said promoter is a differential promoter.

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51. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 7 operably linked to a promoter.
52. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 13 operably linked to a promoter.
53. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 14 operably linked to a promoter.
54. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 18 operably linked to a promoter.
55. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 22 operably linked to a promoter.
56. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for an MHC or HLA.
57. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for a cytokine.
58. The expression vector of claim 57, wherein said cytokine is an interleukin.

59. The expression vector of claim 58, wherein said interleukin is IL-2.
60. The expression vector of claim 58, wherein said interleukin is IL-4.
61. The expression vector of claim 47, further comprising a bacterial or viral genome or portion thereof.
62. The expression vector of claim 61, wherein said viral genome vaccinia virus DNA and said bacterial genome or portion thereof in BCG DNA.
63. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for a tumor rejection antigen precursor, and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor, and (b) a vector containing a nucleic acid sequence which codes for an interleukin.
64. Isolated tumor rejection antigen precursor.
65. Isolated human tumor rejection antigen precursor.

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66. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is mage-1.
67. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is a precursor for antigen F.
68. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 2.
69. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 12.
70. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 13.
71. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 22.
72. Isolated tumor rejection antigen.
73. Isolated human tumor rejection antigen.
74. Isolated tumor rejection antigen of claim 72 having amino acid sequence of SEQ ID NO: 4.
75. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen E.

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76. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen F.
77. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a tumor rejection antigen precursor which provokes an immune response when administered to a subject.
78. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a peptide fragment derived from a tumor rejection antigen precursor, wherein said fragment is larger than the tumor rejection antigen derived from said tumor rejection antigen precursor and smaller than said tumor rejection antigen precursor and which provokes an immune response when administered to a subject.
79. Vaccine of claim 77, wherein said TRAP is a human TRAP.
80. Vaccine of claim 77 wherein said precursor is mage-1.
81. Vaccine of claim 79, wherein said precursor is antigen F precursor.

82. Vaccine useful in treating a patient with a cancer comprising a tumor rejection antigen of claim 72 which provokes an immune response when administered to a subject.
83. Vaccine of claim 82, wherein said tumor rejection antigen has amino acid sequence of SEQ ID NO: 4.
84. The vaccine of claim 81, wherein said tumor rejection antigen is antigen E.
85. The vaccine of claim 81, wherein said tumor rejection antigen is antigen F.
86. The vaccine of claim 77, wherein said tumor rejection antigen precursor is the expression product of an expression vector containing a viral genome or portion thereof.
87. Vaccine useful in treating a patient with a cancer comprising the transfected bacterial of claim 45 and a pharmaceutically acceptable adjuvant.
88. Vaccine useful in treating a cancerous condition comprising the mutated virus of claim 46, and a pharmacologically acceptable adjuvant.

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89. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a complex of a tumor rejection antigen and an HLA molecule.
90. Isolated peptide useful in treating a subject afflicted with a cancerous condition, said peptide having the amino acid of SEQ ID NO: 26.
91. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 27 and a pharmacologically acceptable adjuvant.
92. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 37 and a pharmacologically acceptable adjuvant.
93. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen precursor specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
94. Composition of matter of claim 93, wherein said cell line is a human cell line.

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95. Composition of matter of claim 93, wherein said pharmaceutically acceptable carrier is a liposome.
96. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
97. Composition of matter of claim 96, wherein said cell line is a human cell line.
98. Composition of matter of claim 96, wherein said pharmaceutically acceptable carrier is a liposome.
99. Composition of matter useful in treating a cancerous condition, comprising (i) a tumor rejection antigen or tumor rejection antigen precursor, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.
100. Composition of matter of claim 99, wherein said pharmaceutically acceptable carrier is a liposome.
101. Antibody which specifically binds to a tumor rejection antigen precursor.

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102. Antibody of claim 101, wherein said antibody is a monoclonal antibody.
103. Antibody of claim 101, wherein said tumor rejection antigen precursor is mage-1.
104. Antibody of claim 103, wherein said antibody is a monoclonal antibody.
105. Antibody of claim 101, wherein said tumor rejection antigen precursor is antigen F precursor.
106. Antibody of claim 105, wherein said antibody is a monoclonal antibody.
107. Antibody of claim 101, wherein said tumor rejection antigen precursor is a MAGE precursor.
108. Antibody of claim 107, wherein said antibody is a monoclonal antibody.
109. Antibody of claim 107, wherein said MAGE precursor is mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.
110. Antibody of claim 109, wherein said antibody is a monoclonal antibody.

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111. Antibody which specifically binds to a tumor rejection antigen.
112. Antibody of claim 111, wherein said antibody is a monoclonal antibody.
113. Antibody of claim 111, wherein said tumor rejection antigen is that set forth in SEQ ID NO: 4.
114. Antibody of claim 113, wherein said antibody is a monoclonal antibody.
115. Antibody of claim 111, wherein said tumor rejection antigen is antigen E.
116. Antibody of claim 115, wherein said antibody is a monoclonal antibody.
117. Antibody of claim 111, wherein said tumor rejection antigen is antigen F.
118. Antibody of claim 117, wherein said antibody is a monoclonal antibody.
119. Antibody which specifically binds to a complex of (i) tumor rejection antigen and (ii) HLA molecule, but does not bind to (i) or (ii) alone.

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120. The antibody of claim 119, wherein said antibody is a monoclonal antibody.
121. Method for diagnosing a cancerous condition in a subject, comprising contacting a lymphocyte containing sample of said subject to a cell line transfected with a DNA sequence coding for a tumor rejection antigen precursor expressed by cells associated with said cancerous condition, and determining lysis of said transfected cell line by a cytotoxic T cell line specific for a tumor rejection antigen derived from said tumor rejection antigen precursor, said lysis being indicative of said cancerous condition.
122. Method of claim 121, wherein said tumor rejection antigen precursor is a MAGE antigen.
123. Method for determining regression, progression or onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) tumor rejection antigen precursor, (ii) tumor rejection antigen and (iii) cytolytic T cells specific for a tumor rejection antigen associated with said cancerous condition, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

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124. Method of claim 123, wherein said sample is a body fluid.
125. Method of claim 123, wherein said sample is a tissue.
126. Method of claim 123, comprising contacting said sample with an antibody which specifically binds with said tumor rejection antigen or tumor rejection antigen precursor.
127. Method of claim 126, wherein said antibody is labelled with a radioactive label or an enzyme.
128. Method of claim 126, wherein said antibody is a monoclonal antibody.
129. Method of claim 123, comprising amplifying RNA which codes for said tumor rejection antigen precursor.
130. Method of claim 129, wherein said amplifying comprises carrying out polymerase chain reaction.
131. Method of claim 123, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said tumor rejection antigen precursor.
132. Method of claim 123, comprising assaying said sample for shed tumor rejection antigen.

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133. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for a cytolytic T cell specific for a tumor rejection antigen, presence of said cytolytic T cell being indicative of said cancerous condition."

134. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) removing a lymphocyte containing sample from said subject,

(ii) contacting the lymphocyte containing sample to a cell line transfected with a gene coding for and expressing a gene for a tumor rejection antigen precursor expressed by cancer cells associated with said conditions, under conditions favoring production of cytotoxic T cells against a tumor rejection antigen derived from said tumor rejection antigen precursor, and

(iii) introducing said cytotoxic T cells to said subject in an amount sufficient to lyse said cells.

135. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) identifying a MAGE gene expressed by cancer cells associated with said condition;

(ii) identifying an HLA molecule which presents a portion of an expression product of said MAGE gene;

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(iii) transfecting a host cell having the same HLA molecule as identified in (ii) with said MAGE gene;

(iv) culturing said transfected cells to express said MAGE-gene, and;

(v) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

136. Method of claim 135, wherein said immune response comprises a B-cell response.

137. Method of claim 135, wherein said immune response is a T-cell response.

138. Method of claim 136, wherein said B cell response comprises production of antibodies specific to said tumor rejection antigen or tumor rejection antigen precursor.

139. Method of claim 137, wherein said T-cell response comprises generation of cytolytic T-cells specific for cells presenting said tumor rejection antigen.

140. Method of claim 139, further comprising treating said cells to render them non-proliferative.

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141. Method for treating a subject with a cancerous condition, comprising:

(i) identifying a MAGE gene expressed by said tumor;

(ii) transfecting a host cell having the same HLA type as said patient with said MAGE gene;

(iii) culturing said transfected cells to express said MAGE gene, and;

(iv) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

142. Method of claim 141, further comprising treating said cells to render them non proliferative.

143. Method for treating a subject with a cancerous condition, comprising administering to said subject an amount of a cell transfected with (i) a nucleic acid sequence which codes for a tumor rejection antigen precursor (TRAP) and (ii) a nucleic acid sequence which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said TRAP, wherein said tumor rejection antigen is presented by cells associated with said cancerous condition, sufficient to alleviate said cancerous condition.

144. Method of claim 143, further comprising treating said cell to render it non-proliferative.

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145. Method for preparing a biological material useful in treating a subject afflicted with a cancerous condition, comprising:

(i) transfecting a host cell with a nucleic acid molecule which codes for or expresses a tumor rejection antigen precursor;

(ii) transfecting said host cell with a nucleic acid molecule which codes for an HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor on a cell surface, and;

(iii) treating said host cells under conditions favoring expression of said nucleic acid molecules, and presentation of said tumor rejection antigen by said human leukocyte antigen.

146. Method of claim 145, further comprising treating said host cells to render them non proliferative following presentation of said tumor rejection antigen.

147. Method of claim 146, further comprising transfecting said host cell with a nucleic acid molecule which codes for or expresses a cytokine.

148. Method of claim 146, wherein said cytokine is an interleukin.

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149. Method of claim 146, wherein said human leukocyte antigen is HLA-A1.
150. Method of claim 148, wherein said interleukin is IL-2.
151. Method of claim 146, wherein said interleukin is IL-4.
152. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an amount of a reagent consisting essentially of non-proliferative cell having expressed on its surface a tumor rejection antigen characteristic of cancerous cells in an amount sufficient to elicit an immune response thereto.
153. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a tumor rejection antigen expressed on a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.
154. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a

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tumor rejection antigen precursor expressed by a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.

155. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject a biological sample prepared in accordance with claim 142 in an amount sufficient to alleviate said cancerous condition.
156. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 77 in an amount sufficient to prevent onset of said cancerous condition in said subject.
157. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 78 in an amount sufficient to prevent onset of said cancerous condition in said subject.
158. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 82 in an amount sufficient to prevent onset of said cancerous condition in said subject.

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159. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 86 in an amount sufficient to prevent onset of said cancerous condition in said subject.
160. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 87 in an amount sufficient to prevent onset of said cancerous condition in said subject.
161. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 88 in an amount sufficient to prevent onset of said cancerous condition in said subject.
162. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.
163. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.

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164. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 90 in an amount sufficient to prevent onset of said cancerous condition in said subject.

165. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) identifying cells from said subject which express a tumor rejection antigen precursor and present a tumor rejection antigen derived from said precursor on their surface;

(ii) isolating a sample of said cells;

(iii) cultivating said cell, and;

(iv) introducing said cells to said subject in an amount sufficient to provoke an immune response against said cells.

166. Method of claim 165, further comprising rendering said cells non proliferative, prior to introducing them to said subject.

167. Method for identifying a cytotoxic T cell useful in treating a subject afflicted with a cancerous condition, comprising:

(i) identifying a tumor rejection antigen presented by cells associated with said cancerous condition derived from a tumor rejection antigen

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precursor expressed by said cells, prior to introducing them to said subject;

(ii) contacting a cell presenting said antigen to a cytotoxic T cell, and;

(iii) measuring a parameter selected from the group consisting of (i) proliferation of said cytotoxic T cell and (ii) release of a cytotoxic T cell produced factor, wherein increase in said parameter is indicative of said cancerous condition.

168. Method of claim 167, wherein said factor is tumor necrosis factor.

169. Method for following progress of a therapeutic regime designed to alleviate a cancerous condition, comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) tumor rejection antigen, (ii) a cytolytic T cell specific for cells presenting said tumor rejection antigen, and (iii) an antibody which specifically binds to said tumor rejection antigen at a first time period;

(b) assaying level of the parameter selected in (a) at a second period of time and comparing it to the level determined in (a) as a determination of effect of said therapeutic regime.

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170. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for expression of a TRAP molecule, and comparing levels of expression to a normal level, wherein variance there between is indicative of a cancerous condition.
171. Method of claim 164, comprising measuring expression via polymerase chain reaction.
172. Method of claim 123, comprising intradermally administering an amount of a tumor rejection antigen sufficient to generate a delayed type response in a subject.

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ABSTRACT OF THE DISCLOSURE

The invention relates to an isolated DNA sequence which codes for an antigen expressed by tumor cells which is recognized by cytotoxic T cells, leading to lysis of the tumor which expresses it. Also described are cells transfected by the DNA sequence, and various therapeutic and diagnostic uses arising out of the properties of the DNA and the antigen for which it codes.

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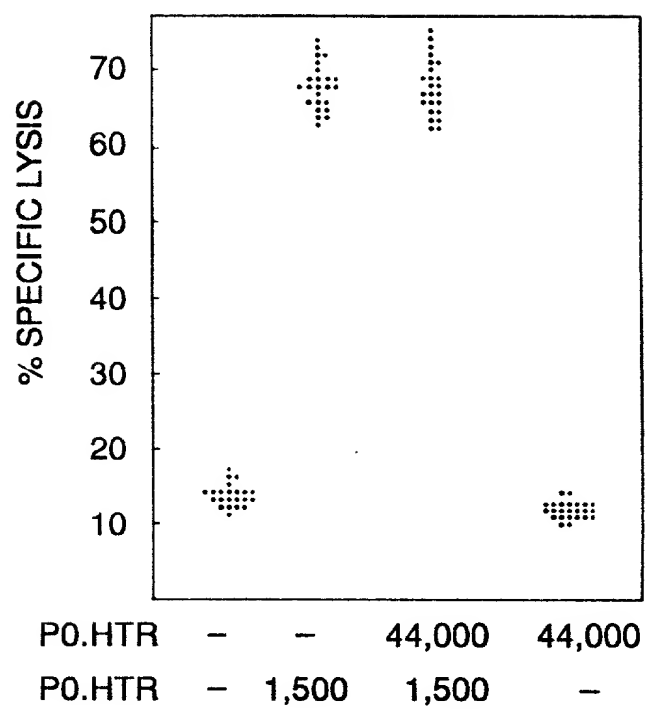
FIG. 1A

FIG. 1B

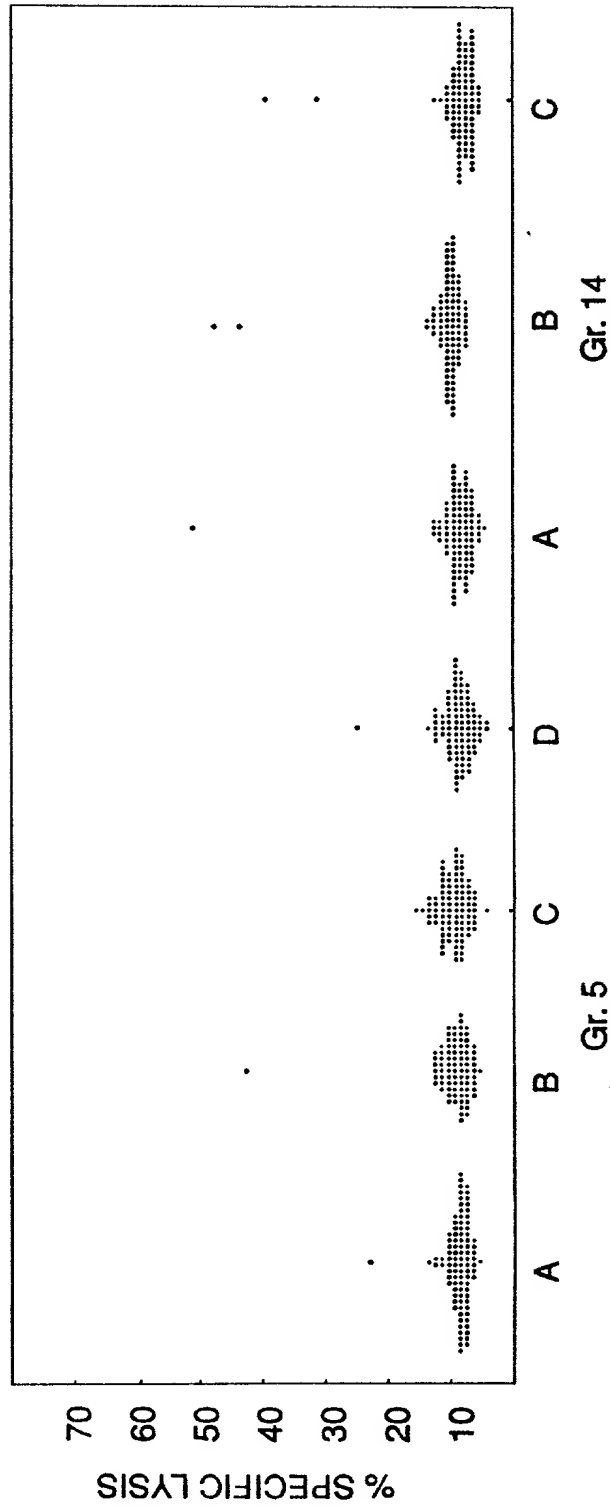


FIG. 2

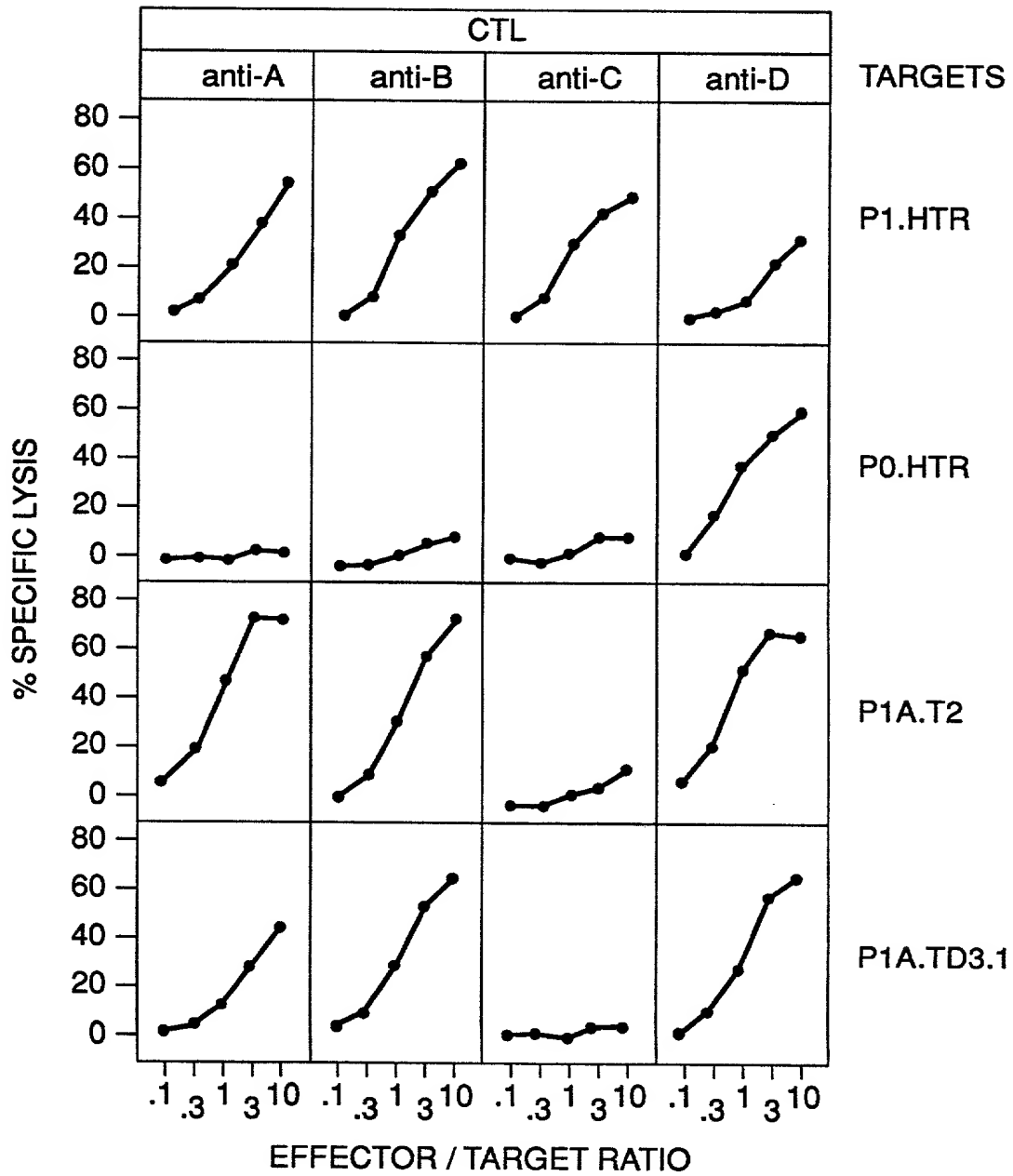
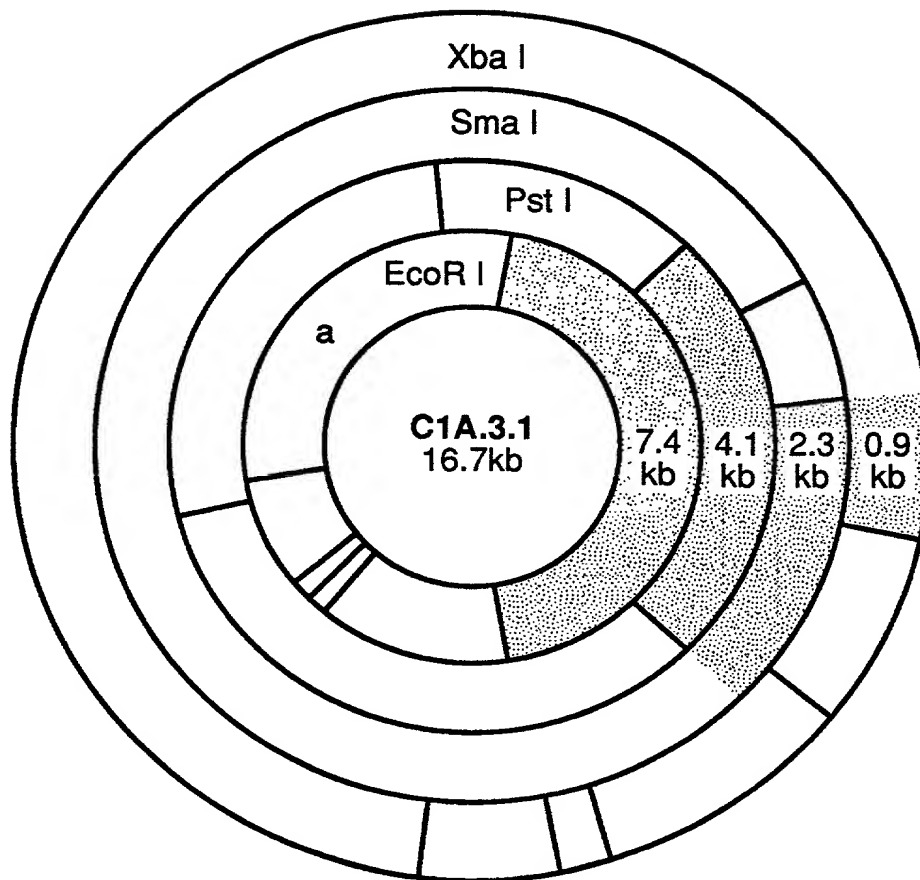


FIG. 3



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FIG. 4

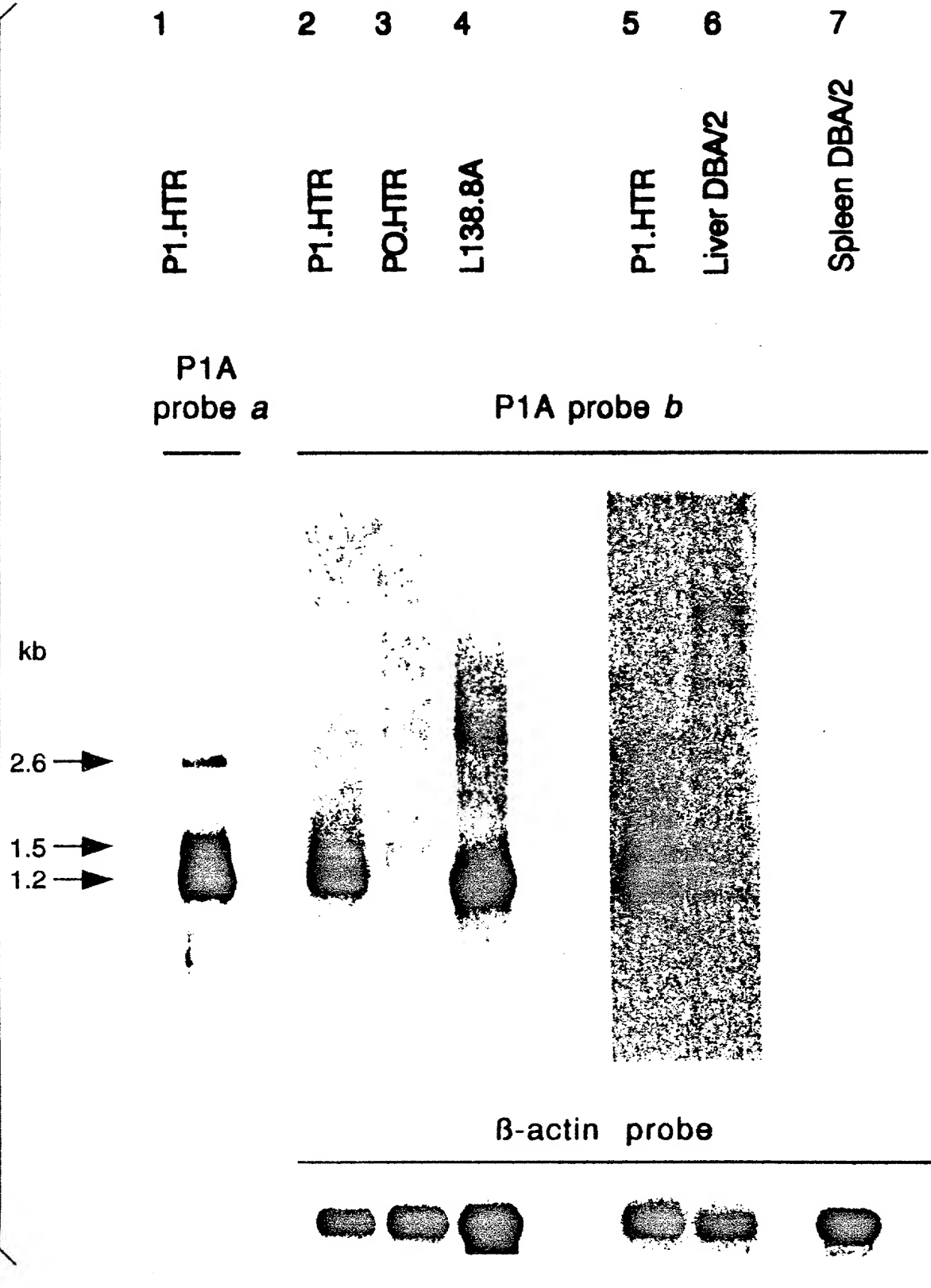


FIG. 5

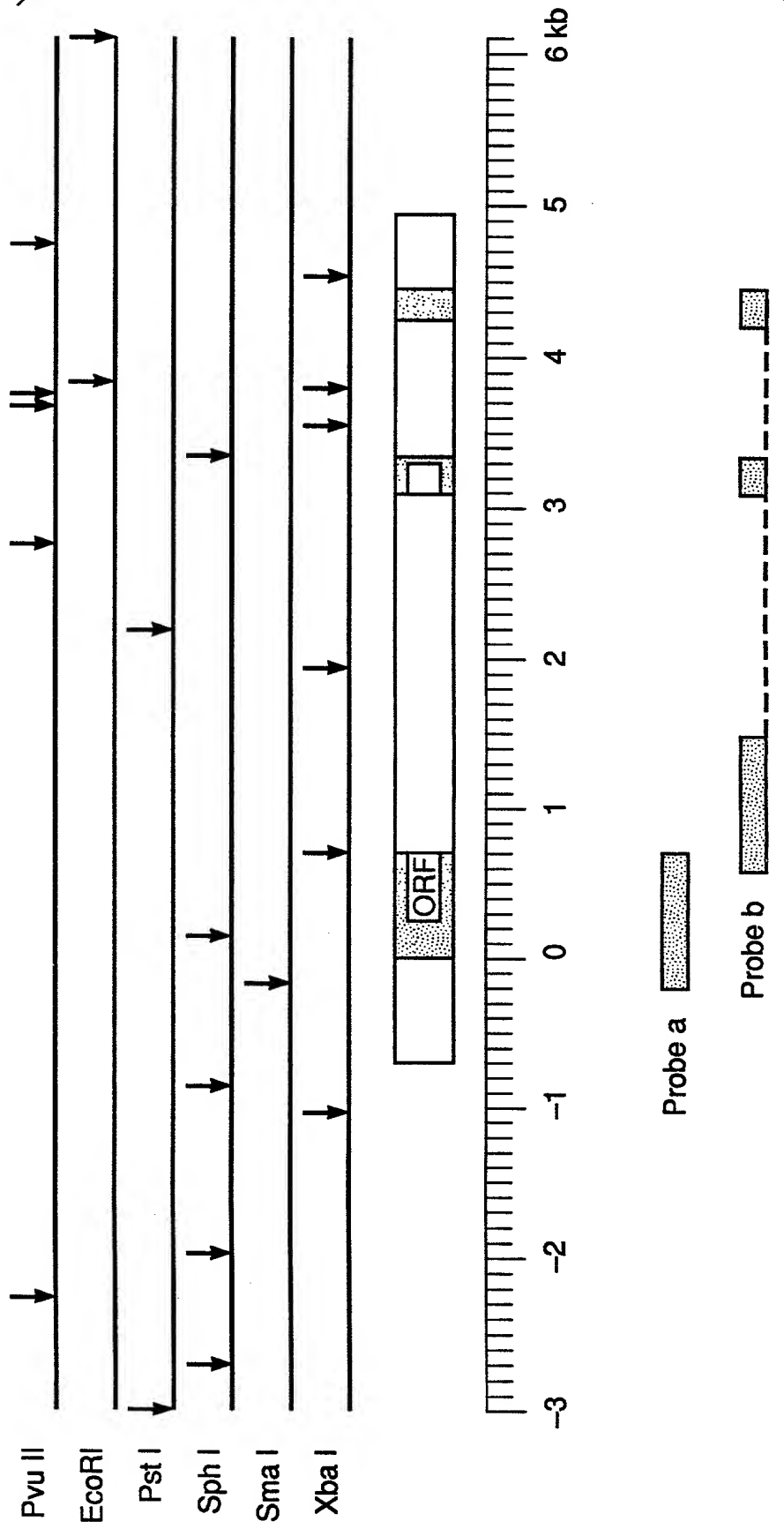
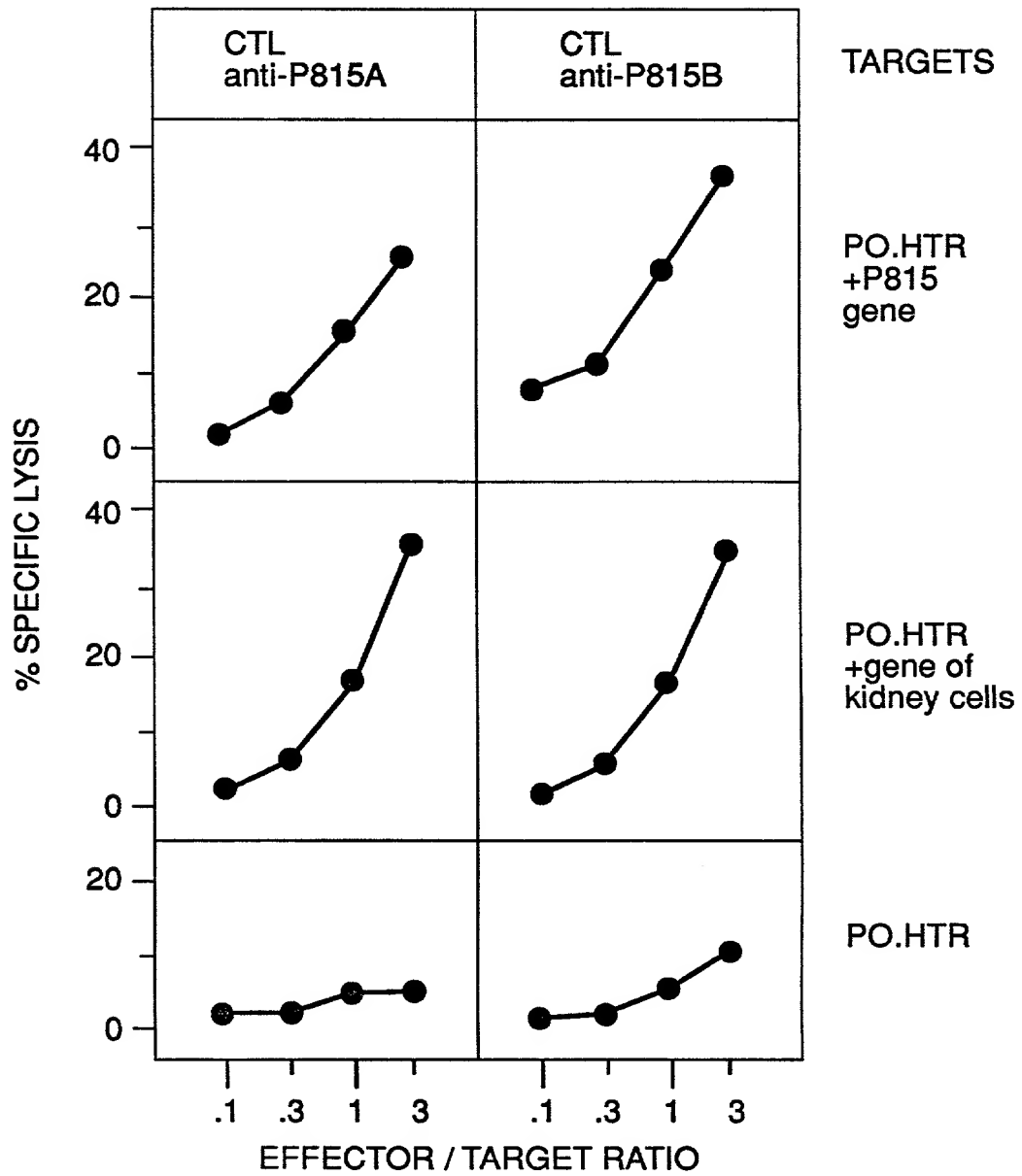


FIG. 6

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FIG. 7

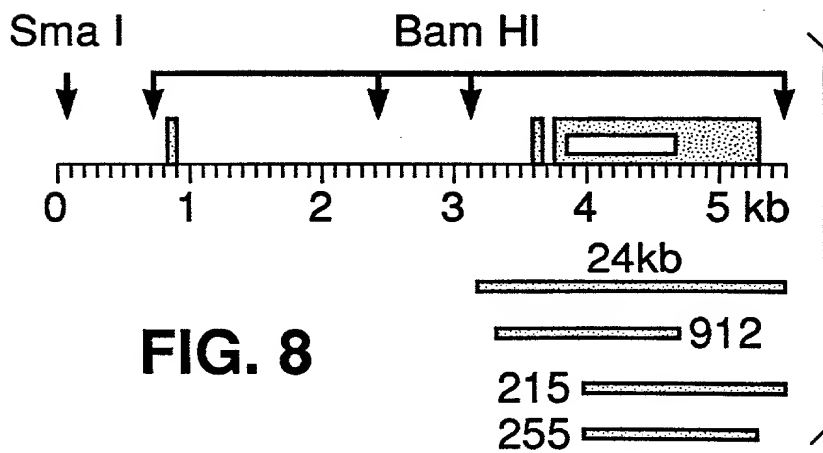
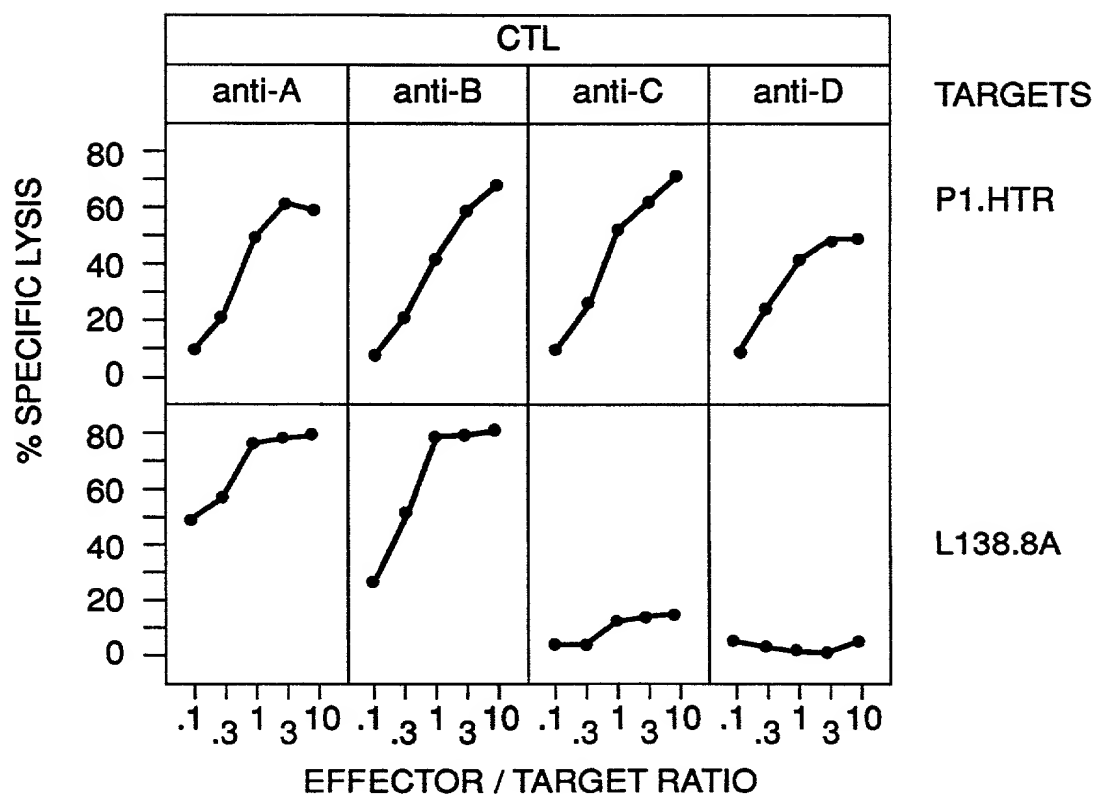


FIG. 8

IMAGE-3 III CCTCCCCAGAGTCTCTCAGGGAGCCTCCagCCtCCCCACTACCATgAACTaCCTCTctgGAGcCAATCCTaTGAGGacTCCAGCAaCCaaGAAGAGGAGG
 IMAGE-2 II CCTCCCCCAcAGTCTCTCAGGGAGCCTCCagCTTctCgACTACCATCAACTaCACTCTtttgGAGaCAATCCgATGAGGGcTCCAGCAaCCaaGAAGAGGAGG
 IMAGE-1 I CCTCCCCAGAGTCTCTCAGGGAGCCTCCGCCtTTCCCACTACCAtCAACtTCACTCGACAGAGGCAACCCAGTGAAGGTTCACAGCAGCCGTGAAGAGGAGG
 225 CHO-8
 III GGCCAAGCACCtccccgaCC-TGGAGTCCgaGTTCCaAGCAGcAcTCAGTAgaAAGGTGGCCgAGTTGGTTcaTTTCTGCTCCTCAAGtATTCGAGGCCA
 II GGCCAAGaAtgTtTcccgacCTTGGAGTCCGAGTTCcaAGCAGCAATCAgTAgaAAGaTGGtTGAGTtGGTTcaTTTCTGCTCCTCAAGtATTCGAGGCCA
 I GGCCAAGcACcCTCTTGATCC-TGGAGTCCtTGTTCCGAGCAGTAATCACTAAGaAGGTGGCTGATTTGGTTGGTTTCTGCTCCTCAAAATATTCGAGGCCA
 325
 III GGGAGCCgGTCACAAAGGCAGAAATGCTGGgGAGTGTCTgTCgAAATtgGcAGtATtctTTTCCCTGTgATCTTCaGCAAAAGCTTccagTTCCTTTCAGCT
 II GGGAGCCgGTCACAAAGGCAGAAATGCTGGAGAGTGTCTcTAGAAATtgCAGgACTtctTTTCCCGtGATCTTCaGCAAAAGCCTCCGAGTaCTTTCAGCT
 I GGGAGCCAGTCACAAAGGCAGAAATGCTGGAGAGTGTcATCAAAATtTCAAGCAGcTGTtTTTCTGAGATCTTCGGCAAGCCtCTGAGTCCtTTCAGCT
 425 SEQ-4
 III GGTCTTTGGCATcGAgCTGATGGAAgTgGACCCCAcCGGCCACTtGTAcATctTTGcCACCTGCCTgGGcCTCTCTACgATGGCCTGCTGGGTGAcAAT
 II GGTCTTTGGCATcGAgGTgTgGAAGtGTCCCCATcAgCCCACTtGTAcATCTCTTGTCAcCTGCCCTgGGcCTCTCTACgATGGCCTGCTGGGcGACAAAT
 I GTCTTTTGGCATtGACGTGAAGGAAGcAGACCCCAcCGGCCACTCTCTATGTCTGTGTcACCTGCCTAGGtCTCTCTATGATGGCCTGCTGGGTGATTAAT
 525
 III CAGATCATGCCCAAGcAGGCCCTCCTGATAATcGTCTGGCCATaTcGGCAAgAGAGGGCGAcTgTGCCcCTTGAGGAGaAAATCTGGGAGGAGCTGATGTG
 II CAGgTCATGCCCAAGcAGGCCCTCCTGATAATcGTCTGGCCATaTcGGCAATaGAGGGCGAcTgTGCCcCTTGAGGAGaAAATCTGGGAGGAGCTGAGTa
 I CAGATCATGCCCAAGcAGGCCCTCCTGATAATtGTCTGGTGTcATGATtTGCAATGGAGGGCGGCCATGTCTCTTGAGGAGGAAATCTGGGAGGAGCTGATGTG
 625 CHO-9

β -action

MAGE

PROBES**FIG. 10**

MZ2-MEL.3.0
 MZ2-MEL 1982
 MZ2-MEL.2.2 E-
 MZ2-PBL-PHA
 Lung
 Kidney

MZ2-MEL 3.0
 MZ2-CTL 82/30
 LB34-MEL
 LB17-MEL
 MI665/2-MEL
 LB41-MEL
 MI10221-MEL
 MI13443-MEL
 SK23-MEL
 SK33-MEL

Other
 melanomas

LB4-MEL
 MI4024-MEL
 MZ3-MEL
 MZ5-MEL
 SK29-MEL
 LB31-COL
 LS411-COL

Other
 tumors

H209-SCLC
 H345-SCLC
 H510-SCLC
 TT

08919669.031797

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FIG. 11

Expression of
antigen MZ2-E
after transaction**

		EXPRESSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:		
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§	
Cells of patient MZ2	melanoma cell line MZ2-MEL3.0	+	++++	++++	++++	+	+	
	tumor sample MZ2 (1982)	+	+++	+++	+++			
	antigen-loss variant MZ2-MEL2.2	+	-	+++	+++	-	-	
	CTL clone MZ2-CTL82/30	-	-	-	-			
	PHA-activated blood lymphocytes	-	-	-	-			
Normal tissues	Liver	-	-	-	-			
	Muscle	-	-	-	-			
	Skin	-	-	-	-			
	Lung	-	-	-	-			
	Brain	-	-	-	-			
	Kidney	-	-	-	-			
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	++++	++++	+	+/-	
	MI6652-MEL	-	-	-	-	-	-	+
	MI10221-MEL	+	-	++	+++	-	-	+
	MI13443-MEL	+	+++	++++	++++	+	+	
	SK33-MEL	+	-	++++	++++	-	-	-
	SK23-MEL	+	-	++++	++++	-	-	+
Melanoma cell lines of other patients	LB17-MEL	+	+	++++	++++	-	-	-
	LB33-MEL	+	-	+++	+++	-	-	-
	LB4-MEL	-	-	-	-	-	-	
	LB41-MEL	-	-	-	-	-	-	
	MI4024-MEL	+	+++	++++	++++	-	-	
	SK29-MEL	-	-	-	-	-	-	
	MZ3-MEL	+	+	++++	++++	-	-	
	MZ5-MEL	+	-	++++	++++	-	-	
Melanoma tumor sample	BB5-MEL	+	+++	++	+++			
Other tumor cell lines	small cell lung cancer H209	+	-	++++	++++			
	small cell lung cancer H345	+	-	++++	++++			
	small cell lung cancer H510	+	-	++++	++++			
	small cell lung cancer LB11	+	+	++++	++++			
	bronchial squamous cell carcinoma LB37	+	-	-	+++			
	thyroid medullary carcinoma TT	+	++++	+++	++++			
	colon carcinoma LB31	+	-	+++	++++	-		
	colon carcinoma LS411	-	-	-	-			
Other tumor samples	chronic myeloid leukemia LLC5	-	-	-	-			
	acute myeloid leukemia TA	-	-	-	-			

* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

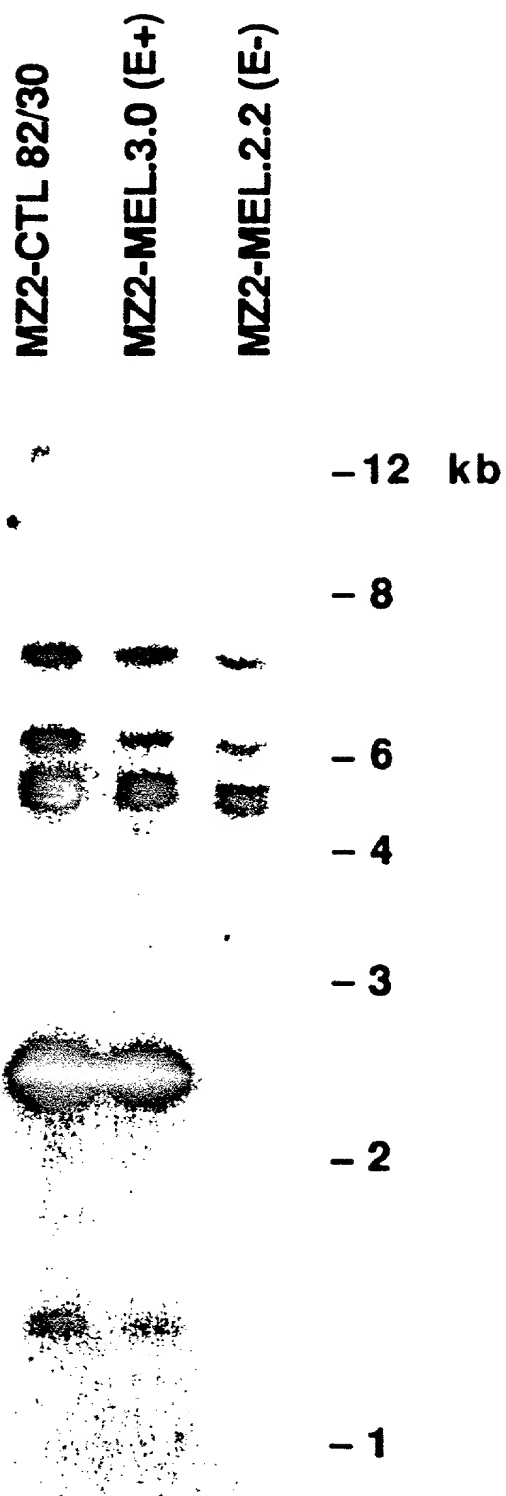
§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

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FIG. 12



2627ED 6956TBB0

FIG. 13

